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(54) Title: MODIFIED OLIGONUCLEOTIDES

(57) Abstract

It is one object of the present invention to provide an oligonucleotide of formula (1): 5'-$(U)_n$-3' in which U is an identical or different radical of a natural or a synthetic nucleoside, wherein the oligonucleotide comprises at least one modified nucleotide dimer comprising two nucleoside analogs connected via an amide-bond that has a certain configuration; the synthesis of these compounds and their use in pharmaceutical preparations.

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Modified oligonucleotides

The present invention relates to modified oligonucleotides comprising at least one nucleotide dimer with a modified backbone, to the modified nucleotide dimers in a certain configuration, processes for the preparation of these oligonucleotides or the nucleotide dimers, the use of these oligonucleotides or the nucleotide dimers and pharmaceutical preparations containing the modified oligonucleotides.

Nucleosides and oligonucleotides have acquired wide interest as antiviral active ingredients or because of their capability to interact with nucleic acids ("antisense" oligonucleotides) and the biological activity associated therewith, see, for example, Uhlmann & Peyman, Chemical Reviews (1990), **90**, 543-584. To provide nucleosides having novel properties or to improve the interaction of antisense oligonucleotides with natural nucleic acids and their stability to nucleases, the sugar radicals of nucleosides (or the nucleotide units in oligonucleotides) or the internucleotide phosphate bond in oligonucleotides have been modified in very different ways.

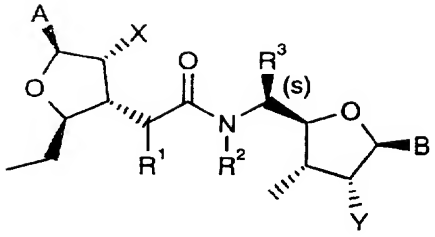
Although several modifications have been performed already, as for example in WO-A-9520597, the importance of a certain configuration at a certain position of the oligonucleotides, and its influence on the hybridization characteristics with DNA/RNA, has not been recognized. Accordingly, the current invention provides oligonucleotides in a certain configuration that are capable of a surprisingly strong hybridization to target RNA or DNA.

Detailed description of the invention

It is one object of the present invention to provide an oligonucleotide of formula 1

$$5'\text{-}(U)_n\text{-}3' \qquad (1)$$

in which U is an identical or different radical of a natural or a synthetic nucleoside, n is an integer from 2 to 200, preferably 2 to 100, more preferred 2 to 50 and most preferred 2 to 20 monomer units; and wherein the oligonucleotide of formula 1 comprises at least one structural unit of formula 2



(2)

wherein

R^1 is H, C_1-C_4alkyl or C_1-C_4alkoxy;
preferred is H or C_1-C_4alkyl;
more preferred is H or methyl;
most preferred is H;

R^2 is H, C_1-C_4alkyl, phenyl, C_1-C_4alkyl-phenyl, C_3-C_9heteroaryl, C_1-C_4alkyl-C_3-C_9heteroaryl or an intercalator; wherein the aryl or heteroaryl is unsubstituted or substituted by OH, R^4, C_1-C_4alkoxy, -O-$(CH_2$-CH_2-O$)_m R^4$, NR^4_2 or NHR^4;
preferred is H, C_1-C_4alkyl, phenyl, C_1-C_4alkyl-phenyl or C_3-C_9heteroaryl;
more preferred is H, methyl, ethyl or phenyl;
most preferred is H, methyl or phenyl;

R^3 is C_1-C_4alkyl, unsubstituted or substituted by OH, NR^4_2 or NHR^4;
preferred is C_1-C_4alkyl;
more preferred is methyl or ethyl;
most preferred is methyl;

R^4 is H or C_1-C_4alkyl;
preferred is methyl or ethyl;
more preferred is methyl;

X and Y are independent of one another, H, OH, OR^4, O-C_1-C_4alkylNHR^4, O-C_1-C_4alkylNR^4_2, -O-$(CH_2$-CH_2-O$)_m R^4$ or -O-CH_2-C(OR^5)H-CH_2-OR^6, -O-CH_2-C(OR^5)H-CH_3;
preferred is H, OH, OR^4, O-C_1-C_4alkylNHR^4, O-C_1-C_4alkylNR^4_2, -O-$(CH_2$-CH_2-O$)_m R^4$;
more preferred is H, OH or OR^4; O-$CH_2CH_2NHR^4$, O-$CH_2CH_2NR^4_2$, O-$CH_2CH_2OR^4$;
even more preferred is H, O-CH_3, O-$CH_2CH_2OCH_3$, O-$CH_2CH_2NHCH_3$, O-$CH_2CH_2N(CH_3)_2$; and
most preferred is H, O-CH_3 and O-$CH_2CH_2OCH_3$;

R^5 is H or C_1-C_{10}alkyl;
preferred is H, CH_3 or C_1-C_4alkyl;
more preferred is H, methyl or ethyl;

R^6 is H, CH_3 or an OH-protecting group;

m is an integer from 1 to 4;
preferred is 1; and

A and B are, independent of one another, a purine or pyrimidine radical or an analogue thereof;

with the proviso that if A and B are thymidine, R^1, R^2 and X are hydrogen and Y is methoxy, R^3 is not methyl.

Beside the presence of one or more structural units of formula (2), the oligonucleotide may be further modified, e.g., by replacement of phosphodiester bonds with -thioate bonds.

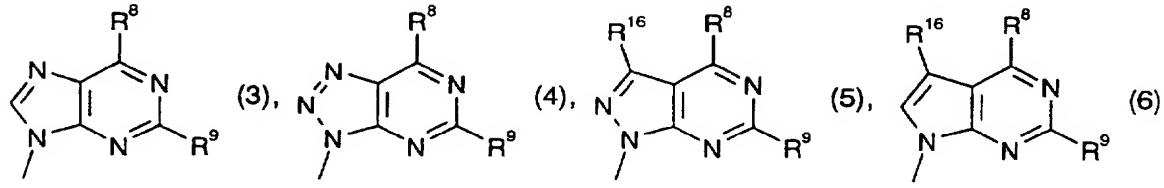
Some examples of alkyl, alkoxy, hydroxyalkyl and aminoalkyl, as used throughout the specification, are methyl, ethyl and the isomers of propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl and dodecyl, and also the corresponding alkoxy, hydroxyalkyl and aminoalkyl radicals. The alkyl, alkoxy, hydroxyalkyl and aminoalkyl radicals preferably contain 1 to 4 C atoms like methyl, ethyl, n- and i-propyl, n-, i- and t-butyl, methoxy, ethoxy, aminomethyl, aminoethyl, hydroxymethyl and hydroxyethyl.

Examples of aminoalkyl are also aminomethyl, aminoethyl, 1-aminoprop-2-yl or -3-yl, 1-aminobut-2-yl or -3-yl or -4-yl, N-methyl- or N,N-dimethyl- or N-ethyl- or N,N-diethyl- or N-2-hydroxyethyl- or N,N-di-2-hydroxyethylaminomethyl or -aminoethyl or -aminopropyl or -aminobutyl. Examples of hydroxyalkyl are hydroxymethyl, 1-hydroxyeth-2-yl, 1-hydroxy-prop-2- or -3-yl, 1-hydroxybut-2-yl, -3-yl or -4-yl.

Examples of C_6-C_{10}aryl are naphthyl and phenyl, wherein phenyl is preferred. The heteroaryl preferably contains 1 to 3 heteroatoms selected from the group consisting of O, S and N, like thienyl, furyl, pyranyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl and pyridazinyl.

A preferred intercalator in connection with the present invention is anthraquinone substituted by a linker, the linker being preferably a chain of 2 to 7 atoms selected from the group consisting of C, N and O, like C_2-C_7alkyl.

If A and/or B is a purine radical or an analogue thereof, it can be a radical of the formula 3, 4, 5 or 6.



in which

R^8 and R^9 independently of one another are H, OH, SH, NH_2, $NHNH_2$, NHOH, NHOalkyl having 1 to 12 C atoms, -N=CH-N(C_1-C_{12}alkyl)$_2$, F, Cl, Br, alkyl or hydroxyalkyl or aminoalkyl or alkoxy or alkylthio having 1 to 12 C atoms, preferably 1 to 4 C atoms; phenyl; benzyl; primary amino having 1 to 20 C atoms, preferably 1 to 12 C atoms and more preferably 1 to 4 C atoms or secondary amino having 2 to 30 C atoms, preferably 2 to 12 C atoms and more preferably 2 to 6 C atoms; and

R^{16} is as defined below.

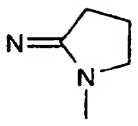
The primary amino preferably contains 1 to 12 and particularly preferably 1 to 6 C atoms, and the secondary amino preferably 2 to 12 and particularly preferably 2 to 6 C atoms.

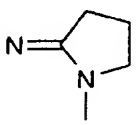
Some examples of alkyl, alkoxy, alkylthio, hydroxyalkyl and aminoalkyl, which preferably contain 1 to 6 C atoms, are methyl, ethyl and the isomers of propyl, butyl, pentyl and hexyl; and also corresponding alkoxy, alkylthio, hydroxyalkyl and aminoalkyl radicals. The alkyl, alkoxy, alkylthio, hydroxyalkyl and aminoalkyl radicals preferably contain 1 to 4 C atoms. Preferred alkyl, alkoxy, alkylthio, hydroxyalkyl and aminoalkyl radicals are methyl, ethyl, n- and i-propyl, n-, i- and t-butyl, methoxy, ethoxy, methylthio and ethylthio, aminomethyl, aminoethyl, hydroxymethyl and hydroxyethyl.

The primary amino and secondary amino can, for example, be radicals of the formula $R^{13}R^{14}N$, in which R^{13} and R^{14} are independently H, C_1-C_{20}alkyl, -aminoalkyl or -hydroxyalkyl, preferably C_1-C_{12}alkyl, -aminoalkyl or -hydroxyalkyl and particularly preferably C_1-C_6alkyl, -aminoalkyl or -hydroxyalkyl; carboxyalkyl or carbalkoxyalkyl, where the carbalkoxy group contains 2 to 8 C atoms and the alkyl group contains 1 to 6, preferably 1 to 4, C atoms; C_2-C_{20}alkenyl, preferably C_2-C_{12}alkenyl and particularly preferably C_2-C_6alkenyl; phenyl, mono- or di(C_1-C_4alkyl- or -alkoxy)phenyl, benzyl, mono- or di(C_1-C_4alkyl- or -alkoxy)benzyl; or 1,2-, 1,3- or 1,4-imidazolyl-C_1-C_6alkyl; or R^{13} and R^{14} together are tetra- or pentamethylene, 3-

oxa-1,5-pentylene, $-CH_2-NR^{15}-CH_2CH_2-$ or $-CH_2CH_2-NR^{15}-CH_2CH_2-$, in which R^{15} is H or C_1-C_4alkyl. The amino group in the aminoalkyl is unsubstituted or substituted by one or two C_1-C_4alkyl or -C_1-C_4hydroxyalkyl groups. The hydroxyl group in hydroxyalkyl is unsubstituted or etherified with C_1-C_4alkyl.

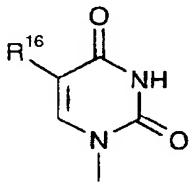
Examples of alkyl have been given previously. Examples of aminoalkyl are aminomethyl, aminoethyl, 1-aminoprop-2-yl or -3-yl, 1-aminobut-2-yl or -3-yl or -4-yl, N-methyl- or N,N-dimethyl- or N-ethyl- or N,N-diethyl- or N-2-hydroxyethyl- or N,N-di-2-hydroxyethylaminomethyl or -aminoethyl or -aminopropyl or -aminobutyl. Examples of hydroxyalkyl are hydroxymethyl, 1-hydroxyeth-2-yl, 1-hydroxyprop-2- or -3-yl, 1-hydroxybut-2-yl, -3-yl or -4-yl. Examples of carboxyalkyl are carboxymethyl, carboxyethyl, carboxypropyl and carboxybutyl, and examples of carbalkoxyalkyl are these carboxyalkyl groups esterified with methyl or ethyl. Examples of alkenyl are allyl, but-1-en-3-yl or -4-yl, pent-3- or 4-en-1-yl or -2-yl, hex-3- or -4- or -5-en-1-yl or -2-yl. Examples of alkyl- and alkoxyphenyl or benzyl are methylphenyl, dimethylphenyl, ethylphenyl, diethylphenyl, methylbenzyl, dimethylbenzyl, ethylbenzyl, diethylbenzyl, methoxyphenyl, dimethoxyphenyl, ethoxyphenyl, diethoxyphenyl, methoxybenzyl, dimethoxybenzyl, ethoxybenzyl, diethoxybenzyl. Examples of imidazolylalkyl, in which the alkyl group preferably contains 2 to 4 C atoms, are 1,2-, 1,3- or 1,4-imidazolylethyl or -n-propyl or -n-butyl.
R^{15} is preferably H, methyl or ethyl.

Preferred examples of primary amino and secondary amino are methyl-, ethyl-, dimethyl-, diethyl-, allyl-, mono- or di(1-hydroxyeth-2-yl)-, phenyl- and benzylamino, acetylamino, isobutyrylamino, benzoylamino, phenoxyacetylamino, 4-tert.-butylphenoxyacetylamino, $N=CH-N(CH_3)_2$, $N=CH-N(C_4H_9)_2$, and  .

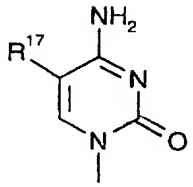
In a preferred embodiment R^8 and R^9 independently of one another are H, F, Cl, Br, OH, SH, NH_2, NHOH, $NHNH_2$, methyl, methylamino, dimethylamino, benzoylamino, isobutyrylamino, methoxy, ethoxy, methylthio, phenoxyacetylamino, 4-tert.-butylphenoxyacetylamino, $N=CH-N(CH_3)_2$, $N=CH-N(C_4H_9)_2$, and  .

Besides purine, some examples of analogues of the purine series are adenine, N-methyladenine, N-benzoyladenine, 2-methylthioadenine, 2-amino-6-chloropurine, 2-amino-6-methylthiopurine, 2-aminopurine, hypoxanthine, 2-aminoadenine, 6-hydroxypurine, guanine and N-isobutyrylguanine. More preferred are adenine, N-methyladenine, N-benzoyladenine, 2-methylthioadenine, 2-aminoadenine, 2-hydroxypurine, 2-amino-6-chloropurine, 2-amino-6-methylthiopurine, guanine, N-isobutyrylguanine, 2-aminopurine and hypoxanthine. Adenine, 2-aminoadenine, 2-aminopurine, guanine and hypoxanthine are particularly preferred.

If A or B in formula 2 is an analogous pyrimidine radical, it is preferably uracil, thymine or cytosine radicals of formulae 9 or 10



(9),



(10),

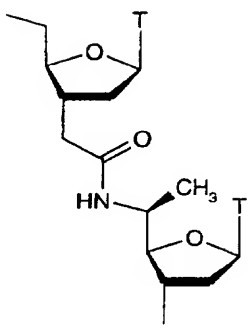
in which R^{16} and R^{17} independently of one another are is H, F, Cl, Br, $CONH_2$, alkyl, propinyl or hydroxyalkyl or aminoalkyl or alkoxy or alkylthio having 1 to 12 C atom; phenyl; benzyl; primary amino having 1 to 20 C atoms or secondary amino having 2 to 30 C atoms; the hydrogen atoms of the NH_2 group in formula 10 are unsubstituted or substituted by C_1-C_6alkyl, benzoyl or benzyl; and the dihydro derivatives of the radicals of formulae 9 and 10:

R^{16} is preferably H, F, Cl, Br, C_1-C_6alkyl, C_1-C_6alkenyl, C_1-C_6alkinyl, C_1-C_6hydroxyalkyl, C_1-C_6aminoalkyl, NHC_1-C_4alkyl, $N(C_1$-C_4alkyl$)_2$, propinyl; and more preferably H, F, Cl, Br, methyl, ethyl, or propinyl; and most preferably H, propinyl or methyl;

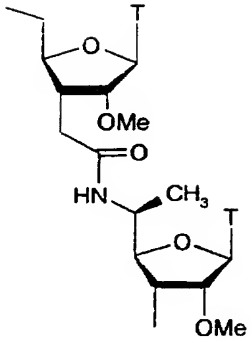
R^{17} is preferably H, F, Cl, Br, C_1-C_6alkyl or C_1-C_6alkoxy, C_1-C_6hydroxyalkyl, C_1-C_6aminoalkyl, NH_2, NHC_1-C_4alkyl, $N(C_1$-C_4alkyl$)_2$, and propinyl; and more preferably H, F, Cl, Br, methyl, ethyl, and propinyl; and most preferably H, propinyl or methyl.

Some examples of pyrimidine analogues are uracil, thymine, cytosine, 5-fluorouracil, 5-chlorouracil, 5-bromouracil, 5-methylcytosine, 5-propinyluracil, 5-propinylcytosine and their base protected derivatives.

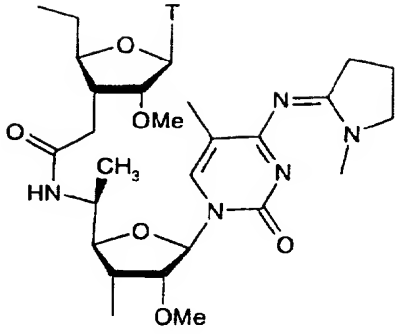
Especially preferred structural units are of formula B8, B28 and B49.



B8;

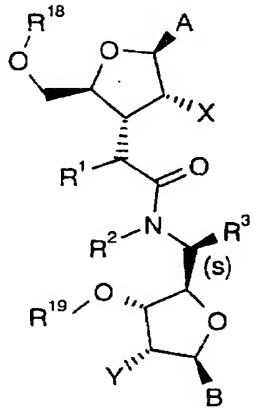


B28;



B49

Another object of the present invention is a nucleoside dimer of the formula 12, that can be used, for example, as a building block for the construction of oligonucleotides as shown in formula 1.



(12)

wherein

R^1, R^2, R^3, X,Y, m, A and B are as defined above;

R^{18} and R^{19} independent of one another are H, an OH-protecting group or a phosphorus-containing, nucleotide-bridge-group-forming radical.

In a preferred embodiment R^{18} is H or an OH-protecting group and R^{19} is a phosphorus-containing, nucleotide-bridge-group-forming radical.

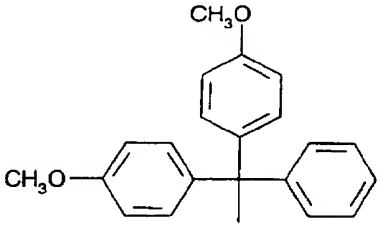
Suitable Protective groups and processes for derivatisation of the hydroxyl groups with such protective groups are generally known in sugar and nucleotide chemistry and described, for example, by B. T. Greene, Protective Groups in Organic Synthesis, Wiley Interscience, New York (1991). Examples of such protective groups are: linear or branched C_1-C_8alkyl, particularly C_1-C_4alkyl, for example methyl, ethyl, n- and i-propyl, n-, i- and t-butyl; C_7-

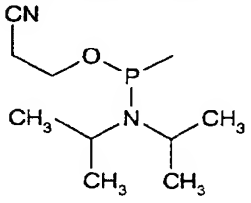
C_{18}aralkyl, for example benzyl, methylbenzyl, dimethylbenzyl, methoxybenzyl, dimethoxybenzyl, bromobenzyl, diphenylmethyl, di(methylphenyl)methyl, di(dimethylphenyl)methyl, di(methoxyphenyl)methyl, di(dimethoxyphenyl)methyl, trityl, tri(methylphenyl)methyl, tri(dimethylphenyl)methyl, methoxyphenyl(diphenyl)methyl, di(methoxyphenyl)phenylmethyl, tri(dimethoxyphenyl)methyl, tri(methoxyphenyl)methyl; triphenylsilyl, alkyldiphenylsilyl, dialkylphenylsilyl and trialkylsilyl having 1 to 20, preferably 1 to 12 and particularly preferably 1 to 8, C atoms in the alkyl groups, for example trimethylsilyl, triethylsilyl, tri-n-propylsilyl, i-propyldimethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, n-octyldimethylsilyl, (1,1,2,2-tetramethylethyl)dimethylsilyl; -(C_1-C_8alkyl)$_2$Si-O-Si(C_1-C_8alkyl)$_2$-, in which alkyl, for example, is methyl, ethyl, n- or i-propyl, n-, i- or t-butyl; C_2-C_{12}acyl, particularly C_2-C_8acyl, for example acetyl, propanoyl, butanoyl, pentanoyl, hexanoyl, benzoyl, methoxybenzoyl, methylbenzoyl, chlorobenzoyl and bromobenzoyl; R^{12}-SO_2-, in which R^{12} is C_1-C_{12}alkyl, particularly C_1-C_6alkyl, C_5- or C_6cycloalkyl, phenyl, benzyl, C_1-C_{12}alkylphenyl and particularly C_1-C_4alkylphenyl, or C_1-C_{12}alkylbenzyl and particularly C_1-C_4alkylbenzyl, or halophenyl or halobenzyl, for example methyl-, ethyl-, propyl-, butyl-, phenyl-, benzyl-, p-bromo-, p-methoxy- or p-methylphenylsulfonyl; unsubstituted or F-, Cl-, Br-, C_1-C_4alkoxy-, tri(C_1-C_4alkyl)silyl- or C_1-C_4alkylsulfonyl-substituted C_1-C_{12}alkoxycarbonyl, preferably C_1-C_8alkoxycarbonyl, for example methoxy-, ethoxy-, n- or i-propoxy- or n-, i- or t-butoxycarbonyl, 2-trimethylsilylethoxycarbonyl, 2-methylsulfonylethoxycarbonyl, or phenoxycarbonyl or benzyloxycarbonyl which is unsubstituted or substituted as for alkoxycarbonyl, for example methyl- or methoxy- or chlorophenoxycarbonyl or -benzyloxycarbonyl, and also 9-fluorenylmethyloxycarbonyl.

If the protecting group is alkyl, it can be substituted by F, Cl, Br, C_1-C_4alkoxy, phenoxy, chlorophenoxy, methoxyphenoxy, benzyloxy, methoxybenzyloxy or chlorophenoxy.

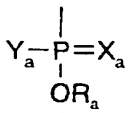
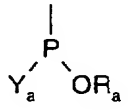
In a preferred embodiment, the protective groups are, independently of one another, linear or branched C_1-C_4alkyl, C_7-C_{18}aralkyl, trialkylsilyl having 1 to 12 C atoms in the alkyl groups; -(C_1-C_4alkyl)$_2$Si-O-Si(C_1-C_4alkyl)$_2$ like $(CH_3)_2$Si-O-Si$(CH_3)_2$- and -(i-C_3H_7)$_2$Si-O-Si(iC_3H_7)$_2$-; C_2-C_8acyl, R^{12}-SO_2-, in which R^{12} is C_1-C_6alkyl; phenyl or benzyl unsubstituted or substituted with F, Cl or Br; C_1-C_4alkylphenyl; C_1-C_4alkylbenzyl; C_1-C_8alkoxycarbonyl; phenoxycarbonyl; benzyloxycarbonyl or 9-fluorenylmethoxycarbonyl.

In a particularly preferred embodiment, the protective groups are methyl, ethyl, n- or i-propyl, n-, i- or t-butyl; benzyl, methylbenzyl, dimethylbenzyl, methoxybenzyl, dimethoxybenzyl, bromobenzyl; diphenylmethyl, di(methylphenyl)methyl, di(dimethylphenyl)methyl, di(methoxyphenyl)methyl, di(methoxyphenyl)(phenyl)methyl, trityl, tri(methylphenyl)methyl, tri(dimethylphenyl)methyl, tri(methoxyphenyl)methyl, tri(dimethoxyphenyl)methyl; trimethylsilyl, triethylsilyl, tri-n-propylsilyl, i-propyldimethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, n-octyldimethylsilyl, (1,1,2,2-tetramethylethyl)dimethylsilyl, -(i-C_3H_7)$_2$Si-O-Si(i-C_3H_7)$_2$-,-(CH_3)$_2$-Si-O-Si(CH_3)$_2$-; C_1-C_8acyl groups like acetyl, propanoyl, butanoyl, pentanoyl, hexanoyl, benzoyl, methylbenzoyl, methoxybenzoyl, chlorobenzoyl and bromobenzoyl; methyl-, ethyl-, propyl-, butyl-, phenyl-, benzyl-, p-bromo-, p-methoxy- and p-methylphenylsulfonyl; methoxy-, ethoxy-, n- or i-propoxy- or n-, i- or t-butoxycarbonyl, or phenoxycarbonyl, benzyloxycarbonyl, methyl- or methoxy- or chlorophenoxycarbonyl or -benzyloxycarbonyl or 9-fluorenylmethoxycarbonyl.

In an especially preferred embodiment R^{18} is  and

R^{19} is 

A phosphorus-containing, nucleotide-bridge-group-forming radical may correspond to formula P1 or P2

 (P1),  (P2)

wherein

Y_a is hydrogen, C_1-C_{12}alkyl, C_6-C_{12}aryl, C_7-C_{20}aralkyl, C_7-C_{20}alkaryl, -OR_b, -SR_b, secondary amino, O^-M^+ or S^-M^+;

X_a is oxygen or sulfur;

R_a is hydrogen, M^+, C_1-C_{12}alkyl, C_2-C_{12}alkenyl or C_6-C_{12}aryl, or the group R_aO- is N-hetero-aryl-N-yl having 5 ring members and from 1 to 3 nitrogen atoms;
R_b is hydrogen, C_1-C_{12}alkyl or C_6-C_{12}aryl; and
M^+ is Na^+, K^+, Li^+, NH_4^+ or primary, secondary, tertiary or quaternary ammonium;
alkyl, aryl, aralkyl and alkaryl in Y_a, R_a and R_b being unsubstituted or substituted by alkoxy, alkylthio, halogen, -CN, -NO_2, phenyl, nitrophenyl or halophenyl.

Y_a contains as secondary amino preferably from 2 to 12 and especially from 2 to 6 carbon atoms.

The secondary amino may be, for example, a radical of the formula R_cR_dN, wherein R_c and R_d, are independently of one another is C_1-C_{20}-, preferably C_1-C_{12}- and especially C_1-C_6-alkyl; C_1-C_{20}-, preferably C_1-C_{12}- and especially C_1-C_6-aminoalkyl; or C_1-C_{20}-, preferably C_1-C_{12}- and especially C_1-C_6-hydroxyalkyl; carboxyalkyl or carbalkoxyalkyl, the carbalkoxy group containing from 2 to 8 carbon atoms and the alkyl group from 1 to 6, preferably from 1 to 4, carbon atoms; C_2-C_{20}-, preferably C_2-C_{12}- and especially C_2-C_6-alkenyl; phenyl, mono- or di-(C_1-C_4alkyl or C_1-C_4alkoxy)phenyl, benzyl, mono- or di-(C_1-C_4alkyl or C_1-C_4alkoxy)benzyl; or 1,2-, 1,3- or 1,4-imidazolyl-C_1-C_6alkyl, or R_c and R_d together are tetra- or penta-methylene, 3-oxa-1,5-pentylene, -CH_2-NR_e-CH_2CH_2- or -CH_2CH_2-NR_e-CH_2CH_2-, wherein R_e is hydrogen or C_1-C_4alkyl. The amino group in aminoalkyl may be substituted by one or two C_1-C_4alkyl or C_1-C_4hydroxyalkyl groups. The hydroxy group in hydroxyalkyl may be etherified by C_1-C_4alkyl.

Primary, secondary, tertiary and quaternary ammonium for Y_a in connection with the definition of M^+ is to be understood as being an ion of the formula $R_fR_gR_hR_iN^+$, wherein R_f is C_1-C_{20}-, preferably C_1-C_{12}- and especially C_1-C_6-alkyl, C_1-C_{20}-, preferably C_1-C_{12}- and especially C_1-C_6-aminoalkyl, C_1-C_{20}-, preferably C_1-C_{12}- and especially C_1-C_6-hydroxyalkyl; carboxyalkyl or carbalkoxyalkyl, the carbalkoxy group containing from 2 to 8 carbon atoms and the alkyl group from 1 to 6, preferably from 1 to 4, carbon atoms; C_2-C_{20}-, preferably C_2-C_{12}- and especially C_2-C_6-alkenyl; phenyl, mono- or di-(C_1-C_4alkyl or C_1-C_4alkoxy)phenyl, benzyl, mono- or di-(C_1-C_4alkyl or C_1-C_4alkoxy)benzyl; or 1,2-, 1,3- or 1,4-imidazolyl-C_1-C_6alkyl, and R_g, R_h and R_i are each independently of the others hydrogen or have the definition of R_f, or R_f and R_g together are tetra- or penta-methylene, 3-oxa-1,5-pentylene, -

CH_2-NR_e-CH_2CH_2- or -CH_2CH_2-NR_e-CH_2CH_2-, wherein R_e is hydrogen or C_1-C_4alkyl, and R_h and R_i each independently of the other have the definition of R_f. The amino group in aminoalkyl may be substituted by one or two C_1-C_4alkyl or C_1-C_4hydroxyalkyl groups. The hydroxy group in the hydroxyalkyl may be etherified by C_1-C_4alkyl.

Examples of carboxyalkyl are carboxymethyl, carboxyethyl, carboxypropyl and carboxybutyl, and examples of carbalkoxyalkyl are those carboxyalkyl groups esterified by methyl or ethyl. Examples of alkenyl are allyl, but-1-en-3-yl or -4-yl, pent-3- or -4-en-1-yl or -2-yl, hex-3- or -4- or -5-en-1-yl or -2-yl. Examples of alkyl- and alkoxy-phenyl and alkyl- and alkoxy--benzyl are methylphenyl, dimethylphenyl, ethylphenyl, diethylphenyl, methylbenzyl, dimethylbenzyl, ethylbenzyl, diethylbenzyl, methoxyphenyl, dimethoxyphenyl, ethoxyphenyl, diethoxyphenyl, methoxybenzyl, dimethoxybenzyl, ethoxybenzyl and diethoxybenzyl. Examples of imidazolylalkyl in which the alkyl group preferably contains from 2 to 4 carbon atoms are 1,2-, 1,3- or 1,4-imidazolyl-ethyl or -n-propyl or -n-butyl. R_e is preferably hydrogen, methyl or ethyl.

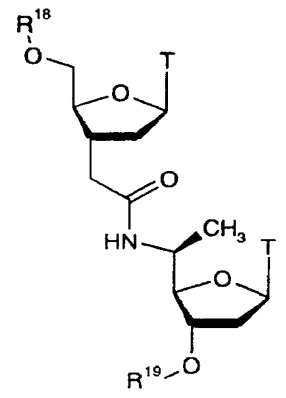
Preferred examples of primary amino and secondary amino are methyl-, ethyl-, dimethyl-, diethyl-, diisopropyl, mono- or di-(1-hydroxy-eth-2-yl)-, phenyl- and benzyl-amino, acetylamino and benzoylamino and piperidinyl, piperazinyl and morpholinyl.

Preferred examples of primary and secondary ammonium are methyl-, ethyl-, dimethyl-, diethyl-, diisopropyl-, mono- or di-(1-hydroxy-eth-2-yl)-, phenyl- and benzyl-ammonium.

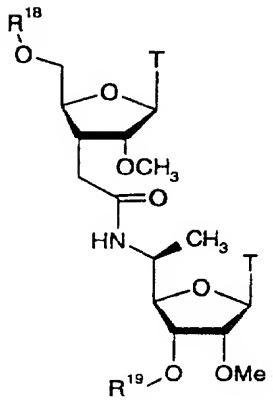
Examples of Y_a, R_a and R_b as alkyl are methyl, ethyl and the isomers of propyl, butyl, pentyl, hexyl, heptyl and octyl; examples of Y_a, R_a and R_b as aryl are phenyl and naphthyl; examples of R_a as alkenyl are allyl and (C_1-C_4alkyl)CH=CH-CH_2-; examples of Y_a as aralkyl are phenyl-C_nH_{2n}- wherein n is a number from 1 to 6, especially benzyl; examples of Y_a as alkaryl are mono-, di- and tri-(C_1-C_4alkyl)phenyl. Preferred substituents are chlorine, bromine, methoxy, -NO_2, -CN, 2,4-dichlorophenyl and 4-nitrophenyl. Examples of R_b are 2,2,2-trichloroethyl, 4-chlorophenyl, 2-chlorophenyl and 2,4-dichlorophenyl; and examples of R_bO- as N-heteroaryl are pyrrol-N-yl, triazol-N-yl and benzotriazol-N-yl.

In an even more preferred form, R_a is β-cyanoethyl and Y_a is di(isopropylamino).

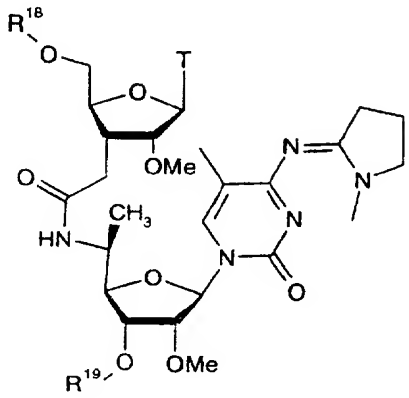
In an especially preferred form the dinucleoside analog is of formula C8, C28 or C49



C8;

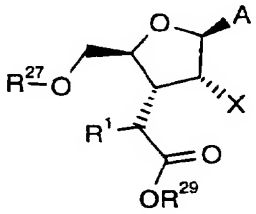


C28,



C49

The invention further relates to a process for the preparation of compounds of the formula 12, which is characterized in that a compound of the formula 14



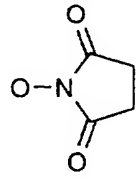
(14)

wherein

R^1, X and A are as defined above; and

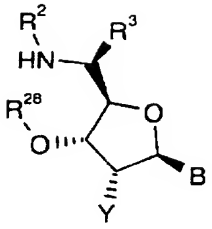
R^{27} is H or an OH-protecting group as defined above; and

R^{29} is H or an ester activating group like C_6F_5, p-NO_2-phenyl, hydroxybenzotriazol-1-yl and



;

is reacted with a compound of the formula 15,



(15)

wherein

R^2, R^3, Y and B are as defined above; and

R^{28} is H, an OH-protecting group as defined above, or a phosphorus-containing, nucleotide-bridge-group-forming radical;

if required (R^{29} = H) in the presence of a condensing agent like, e.g., dicyclohexylcarbodiimide, TBTU (benzotriazol-1-yl-tetramethyluronium tetrafluoroborate) or HBTU (hexafluorophosphate).

Compounds of formulae 14 and 15 can be prepared, for example, according to De Mesmaeker *et al.*, Angew. Chem. Int. Ed. Engl. (1994), **33**, 226-229 or Pudlo & Townsend, Tetrahedron Lett. (1990), **31**, 3101.

The temperature in the synthesis reaction can be from -80 to 150°C, preferably 0 to 100°C.

In general, solvents are used which are protic and/or aprotic, and particularly preferably dipolar. Examples of solvents which can be employed on their own or as a mixture of at least two solvents are ethers (dibutyl ether, tetrahydrofuran, dioxane, diethylene glycol dimethyl ether, ethylene glycol dimethyl or diethyl ether, diethylene glycol diethyl ether, triethylene glycol dimethyl ether), halogenated hydrocarbons (methylene chloride, chloroform, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane), carboxylic acid esters and lactones (ethyl acetate, methyl propionate, ethyl benzoate, 2-methoxyethyl acetate, methoxymethyl acetate, γ-butyrolactone, δ-valerolactone, pivalolactone), carboxamides and lactams (N,N-dimethylformamide, N,N-diethylformamide, N,N-dimethylacetamide, tetramethylurea, hexamethylphosphoramide, γ-butyrolactam, ∈-caprolactam, N-methylpyrrolidone, N-acetylpyrrolidone, N-methylcaprolactam), sulfoxides (dimethyl sulfoxide), sulfones (dimethyl sulfone, diethyl sulfone, trimethylene sulfone, tetramethylene sulfone), tertiary amines (triethylamine, N-methylpiperidine, N-methylmorpholine), aromatic hydrocarbons, for example benzene or substituted benzenes (chlorobenzene, o-dichlorobenzene, 1,2,4-

trichlorobenzene, nitrobenzene, toluene, xylene) and nitriles (acetonitrile, propionitrile, benzonitrile, phenylacetonitrile), and also aliphatic or cycloaliphatic hydrocarbons (pentane, petroleum ether, hexane, cyclohexane and methylcyclohexane).

An object of the present invention is the use of a dimer of formula 12 for the preparation of oligonucleotides which comprise one or more identical or different dimer units of formula 12.

The oligonucleotides according to the invention can be prepared in a manner known per se by various processes, preferably on a solid support. For details see for example Gait, Oligonucleotide Synthesis: A Practical Approach, IRL Press, Oxford (1984).

The oligonucleotides of the formula 1 and the dimeres of formula 12 can be used in a method of treatment. They have, e.g., antiviral and antiproliferative properties. The oligonucleotides and dimeres according to the invention have a surprisingly high stability to degradation by nucleases. A very good pairing with complementary nucleic acid strands, particularly of the RNA type, is also observed. The oligonucleotides according to the invention are therefore particularly suitable for antisense technology, i.e. for inhibition of the expression of undesired protein products due to the binding to suitable complementary nucleotide sequences in nucleic acids (see EP-A-266099, WO-A-8707300 and WO-A-8908146). They can be employed for the treatment of infections and diseases, for example by blocking the expression of bioactive proteins at the nucleic acid stage (for example oncogenes). The oligonucleotides according to the invention are also suitable as diagnostics and can be used as gene probes for the detection of viral infections or of genetically related diseases by selective interaction at the single- or double-stranded nucleic acid stage. In particular - due to the increased stability to nucleases - diagnostic use is not only possible *in vitro* but also *in vivo* (for example tissue samples, blood plasma and blood serum). Use possibilities of this type are described, for example, in WO-A-9106556.

The invention relates to the use of the oligonucleotides according to the invention as diagnostics for the detection of viral infections or of genetically related diseases.

The invention also relates to the oligonucleotides of the formula 1 and dinucleosides of formula 12, according to the invention, for use in a therapeutic process for the treatment of diseases in mammals including humans by means of inactivation of nucleotide sequences

in the body. The dose when administered to mammals of about 70kg body weight can be, for example, 0.01 to 1000mg per day. Administration is preferably effected parenterally, for example intravenously or intraperitoneally, in the form of pharmaceutical preparations.

The invention further relates to a pharmaceutical preparation comprising an effective amount of an oligonucleotide of the formula 1 or dimeres of formula (12) on its own or together with other active ingredients, a pharmaceutical carrier in a customary amount and, if appropriate, excipients.

The pharmacologically active oligonucleotides or dimeres according to the invention can be used in the form of parenterally administrable preparations or of infusion solutions. Solutions of this type are preferably isotonic aqueous solutions or suspensions, it being possible to prepare these before use, for example in the case of lyophilized preparations which contain the active substance on its own or together with a carrier, for example mannitol. The pharmaceutical preparations can be sterilized and/or contain excipients, for example preservatives, stabilisers, wetting and/or emulsifying agents, solubilisers, salts for regulating the osmotic pressure and/or buffers. The pharmaceutical preparations, which if desired can contain further pharmacologically active substances such as, for example, antibiotics, are prepared in a manner known per se, for example by means of conventional dissolving or lyophilizing processes, and contain about 0.1% to 90%, in particular from about 0.5% to about 30%, for example 1% to 5% of active substance(s).

The examples below illustrate the invention.

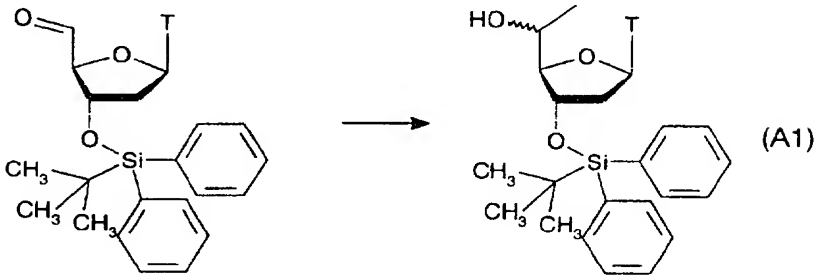
The following abbreviations are used in the examples:

Ac	acetyl
Bn:	benzyl
DMT:	dimethoxy trityl
HV:	high vacuum
Me:	methyl
pMeOBOM	(p-methoxyphenyl)-methoxymethyl
(MeO)Bn	(p-methoxyphenyl)-methyl
nBu_4NF:	tetrabutyl ammonium fluoride
O-Ac:	acetate

Ph:	phenyl
pMeOBOM:	p-methoxybenzyloxybenzyl
RT:	room temperature
T:	thymin-1-yl
$tBuPh_2Si$:	tert. butyldiphenylsilyl
Ts:	p-toluenesulfonyl
TTTr:	tris tert. butyl trityl

A) Preparation of Modified Nucleosides and Dinucleotide Analogs

Example A1: Preparation of compound (A8)

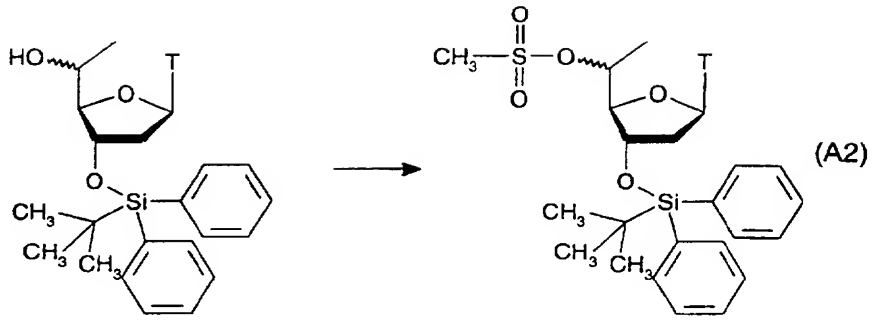


(I) (A1)

For preparation of the aldehyde **I** see: J. Lebreton, A. De Mesmaeker, A. Waldner *Synlett*, **1994**, 54.

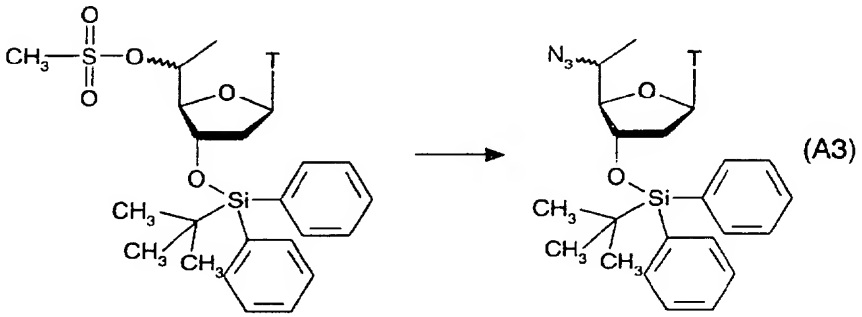
A solution of dry $CeCl_3$ (31.8 g,128.7 mmol) in THF (300 ml) at -78°C is treated with CH_3MgBr (46.8 ml, 3M solution in Et_2O, 140.4 mmol) and stirred for 2.5 h at -78°C. A solution of aldehyde **I** (5.6 g, 11.7 mmol) is added and stirring is continued for 2 h at -78°C. The reaction mixture is poured into a saturated, aqueous solution of $KHSO_4$ and extracted with CH_2Cl_2 (3x). The combined organic layers are washed with Brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 25-50% EtOAc in hexane to give compound **A1** (3.3 g, 56%).

^1H NMR (250 MHz, $CDCl_3$): δ = 6.2 (m, 1H, H-C(1')), mixture of diastereomers.



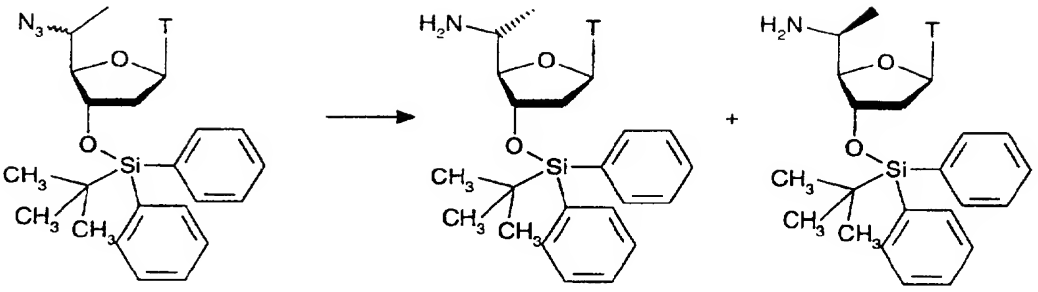
To a solution of compound **A1** (3.0 g, 6.06 mmol) in pyridine (20 ml) is added $MeSO_2Cl$ and the reaction is stirred at 0°C for 1.5 h. The reaction mixture is diluted with CH_2Cl_2 (100 ml), washed with aqueous citric acid and brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (50% EtOAc in hexane) to give compound **A2** (2.16 g, 63%).

^1H NMR (250 MHz, $CDCl_3$): δ = 2.9 (2s, 3H, CH_3SO_2), mixture of diastereomers.
Ms(FD): 573 (M)



To a solution of compound **A2** (2.0 g, 3.48 mmol) in DMF (10 ml) is added NaN_3 (1.704 mg, 26.2 mmol) and the reaction mixture is stirred for 6 h at 65°C. The reaction mixture is poured into a saturated, aqueous solution of NH_4Cl and extracted with EtOAc (3x). The combined organic layers are washed with Brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 35-40% EtOAc in hexane) to give compound **A3** (1.32 g, 72%).

^1H NMR (250 MHz, $CDCl_3$): δ = 6.45 (m, 1H, H-C(1')), mixture of diastereomers.
Ms(CI): 537 (M+NH4)

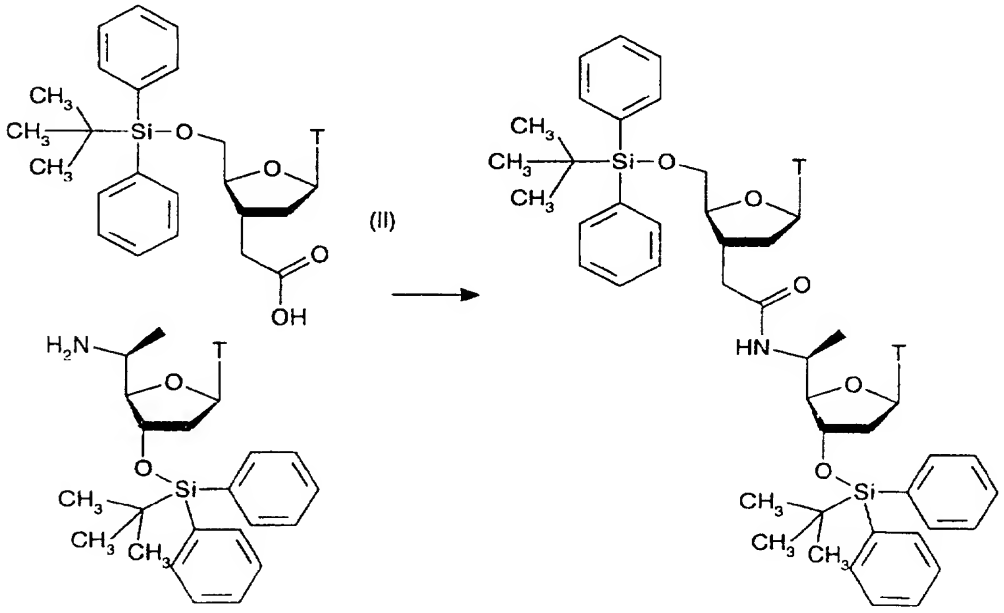


A4 A4a

To a solution of compound **A3** (1.3 g, 2.51 mmol) in MeOH (40 ml) is added $SnCl_2 2\ H_2O$ (2.54 g, 11.3 mmol). The reaction mixture is stirred for 28 h at 25°C. The reaction mixture is neutralized with saturated, aqueous solution of Na_2CO_3 and concentrated. The mixture is diluted with saturated, aqueous solution of Na_2CO_3 and extracted with CH_2Cl_2 (3x). The combined organic layers are washed with Brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 5-10 % MeOH in CH_2Cl_2) to give the two diastereomeric compounds **A4a** (R-C(5') configuration,458.8 mg, 37%) and **A4** (S-C(5'), configuration 133.8 mg, 11 %).

A4a: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.79 dd, 1H, H-C(4')); MS(EI): 494 (M+H)

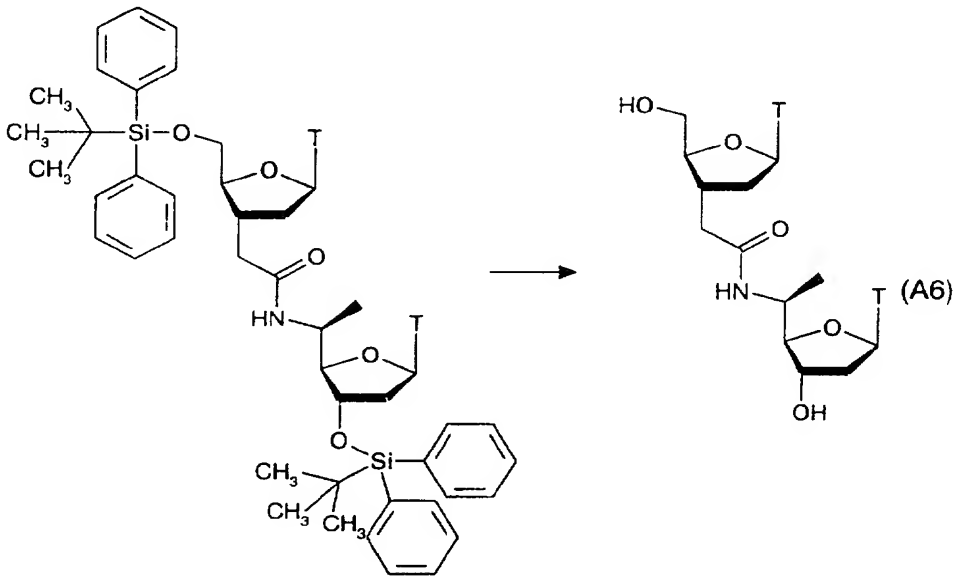
A4: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.70 dd, 1H, H-C(4')); MS(EI): 494 (M+H)



(II) (A5)

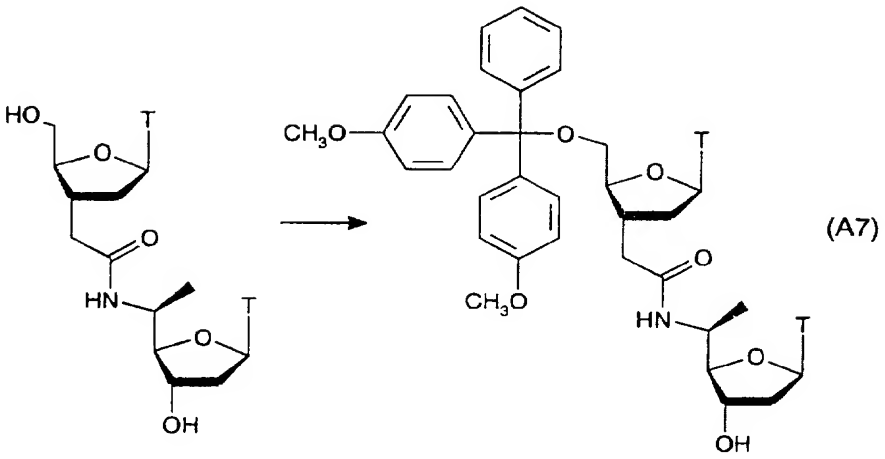
A solution of carboxylic acid **II** (cf. A. De Mesmaeker, A. Waldner, J. Lebreton, P. Hoffmann, V. Fritsch, R. M. Wolf, S. M. Freier, *Angew. Chem. Int. Ed.* **1994**, *33*, 226.) (142 mg, 0.272 mmol, dried over P_2O_5 on HV, 16.0 h) in CH_3CN (2 ml) is treated with Et_3N (30 mg, 0.299 mmol), O-(1-benztriazol-1-yl)-N,N,N,N-tetramethyluroniumtetrafluoroborat (95 mg, 0.299 mmol) and hydroxybenztriazol (18 mg, 0.135 mmol). The reaction mixture is stirred for 2 h. A solution of amine **A4a** (133 mg, 0.271 mmol, dried over P_2O_5 on HV, 16.0 h) in CH_3CN (2 ml) and Et_3N (30 mg, 0.299 mmol) are added to the reaction mixture and stirring is continued for 3 h. The reaction mixture is poured into aqueous, saturated NaH_2PO_4-solution and concentrated. The aqueous phase is extracted with CH_2Cl_2 (3x), the combined organic layers are washed with aqueous, saturated NaH_2PO_4-solution, brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (5% MeOH in CH_2Cl_2) to give compound **A5** (268 mg, 99 %).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.23, 5.58 (2dd, 2H, 2x H-C(1')); MS(EI): 996 (M-H)



A solution of compound **A5** (265 mg, 0.266 mmol) in THF (3 ml) is treated with TBAF (0.58 ml of 1.0 M solution in THF, 0.58 mmol) and stirred at 25°C for 4.5 h. The reaction is concentrated and purified by flash chromatography (10 - 20% MeOH in CH_2Cl_2) to give compound **A6** (123 mg, 89%).

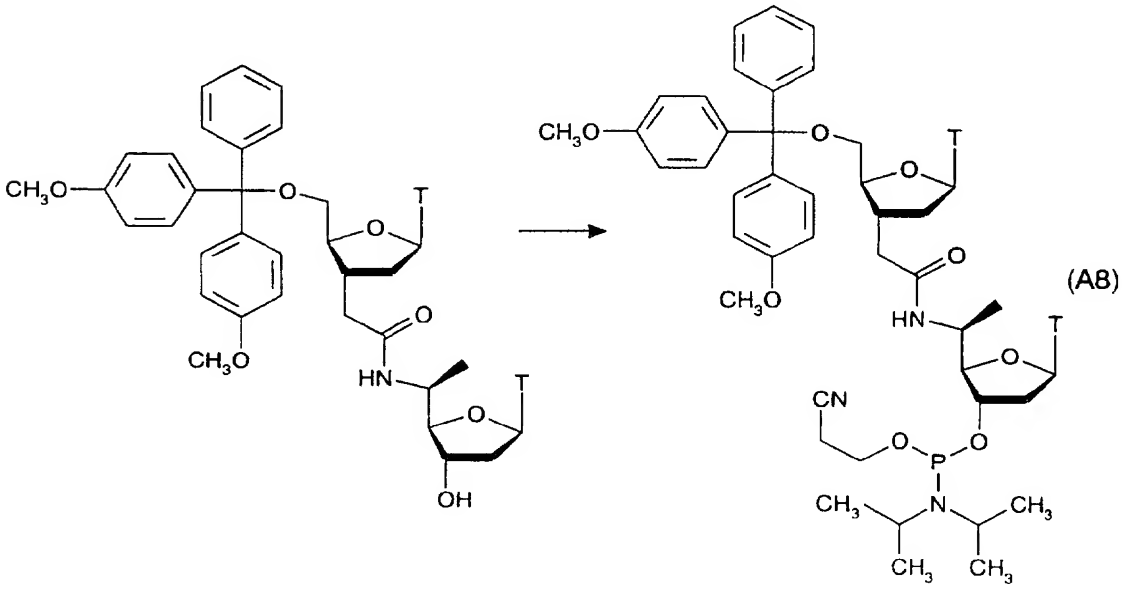
^{1}H NMR (400 MHz, D_6-DMSO): δ = 6.07, 5.94 (2dd, 2H, 2x H-C(1')); MS(EI): 520 (M-H)



(A7)

A solution of compound **A6** (120 mg, 0.230 mmol) in pyridine (3 ml) is treated with 4,4'-dimethoxytriphenylmethylchloride (233 mg, 0.690 mmol) and stirred for 24 h at 25°C. The reaction mixture is poured into aqueous, saturated $NaHCO_3$-solution, extracted with CH_2Cl_2 (3x), the organic layers are washed with brine, dried (Na_2SO_4), concentrated, coevaporated with toluene (3x) and purified by flash chromatography (10-20% MeOH in EtOAc, 1% Et_3N) to give compound **A7** (151 mg, 80 %).

^{1}H NMR (250 MHz, CDCl3): δ = 6.08, 5.85 (2dd, 2H, 2x H-C(1')); MS(EI): 822 (M-H)

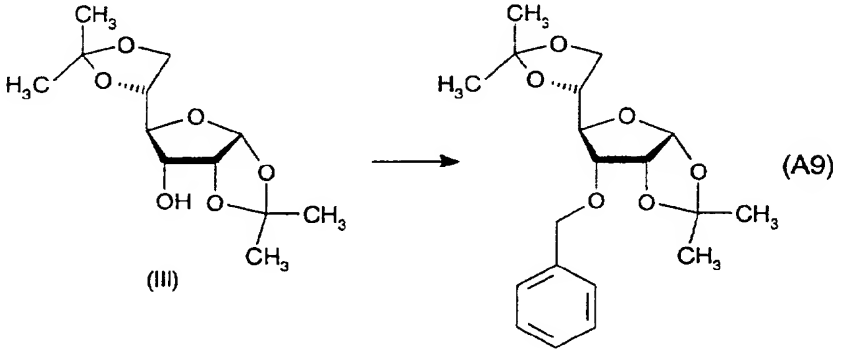


(A8)

Alcohol **A7** (108 mg, 0.130 mmol), dissolved in CH_2Cl_2 (2ml), is added to a solution of di-isopropylammonium tetrazolide (15 mg, 0.088 mmol) and cyanoethoxy-bis-diisopropylamino-phosphine (58 mg, 0.195 mmol) in CH_2Cl_2 (2 ml) at 25°C. The reaction mixture is stirred for 3 h, poured into aqueous, saturated $NaHCO_3$-solution, extracted with CH_2Cl_2 (3x), the organic layers are washed with brine, dried (Na_2SO_4), concentrated, and purified by flash chromatography (1-10 % MeOH in EtOAc, 1% Et_3N) to give compound **A8** (120 mg, 90 %).

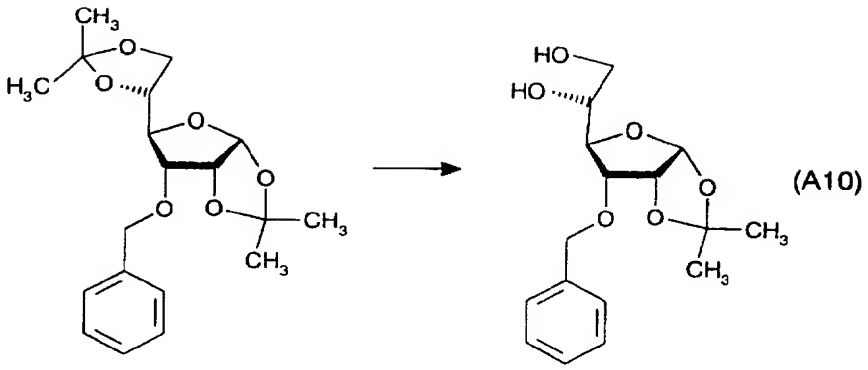
^{31}P NMR (101 MHz, $CDCl_3$): δ = 149.3, 149.0 (2 diastereomers); MS(EI): 1023 (M-H)

Example A2: Preparation of compound (A28)



A solution of compound **III** (cf. D. C. Baker, D. Horton, C.G. Tindal *Methods Carbohydr. Chem.* **1972**, *7*, 3) (47.5 g, 0.182 mol) in THF (70 ml) is added to a suspension of NaH (8.76 g, 55%, 0.201 mol, washed with hexane) in THF (110 ml) at 0°C. The reaction is stirred for 1.0 h at 0°C and 0.5 h at 25°C. Benzylbromide (46.7 g, 0.273 mol) and Bu_4NI (3.36 g, 9.1 mmol) are added to the reaction mixture and stirring is continued for 1.0 h at 25°C. The reaction mixture is poured into a saturated, aqueous solution of NH_4Cl and extracted with EtOAc (3x). The combined organic layers are washed with Brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 20% EtOAc in hexane) to give compound **A9** (55.0 g, 86%)

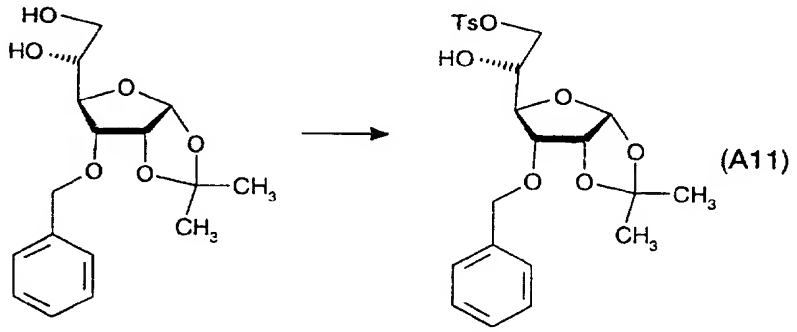
^1H NMR (500 MHz, $CDCl_3$): δ = 1.60, 1.40, 1.38, 1.37 (4s, 12H, CH_3); MS (FD): 350 (M)



(A10)

Compound **A9** (55.0 g, 0.157 mol) is dissolved in AcOH/H_2O (9/1, 1105 ml) and stirred for 2.0 h at 40°C. The reaction mixture is concentrated coevaporated with toluene (3x) and purified by flash chromatography (silica, 65% EtOAc in hexane) to give diol **A10** (29.0 g, 60%).

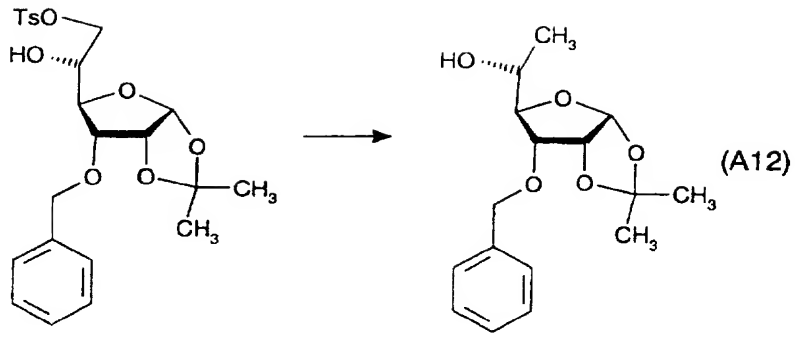
^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.60, 1.37 (2s, 6H, CH_3); MS(FD): 310 (M)



(A11)

A solution of compound **A10** (29.0 g, 93.5 mmol) in pyridine (250 ml) is treated with toluene-4-sulfonyl-chloride (25.0 g, 130.9 mmol) and DMAP (1.1 g, 9.4 mmol) at 0°C. The reaction is stirred for 4.0 h at 25°C, quenched with MeOH (11 ml), stirred for additional 0.3 h, concentrated, coevaporated with toluene (2x) and purified by flash chromatography to give compound **A11** (36.8 g, 85%)

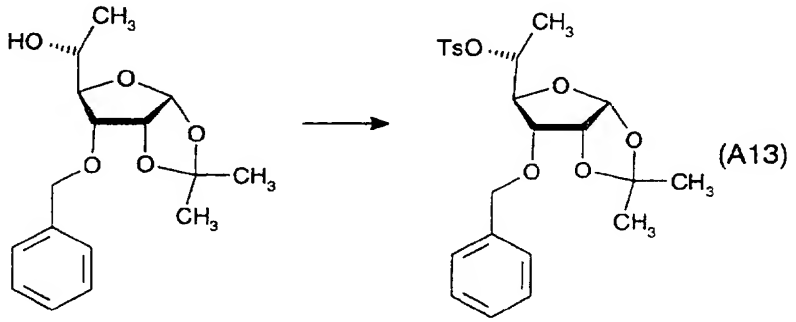
^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.57, 1.35 (2s, 6H, CH_3); MS(FD): 464 (M)



(A12)

A solution of compound **A11** (11.7 g, 25.3 mmol) in DME (83 ml, degassed with Argon) is treated with NaI (11.4 g, 76.0 mmol), Bu_3SnH (11.1 g, 38.0 mmol) and AIBN (410 g, 0.25 mmol) and stirred for 1.0 h at 80°C. The reaction mixture is adsorbed onto silica gel, concentrated and purified by flash chromatography (silica, 30% EtOAc in hexane) to give compound **A12** (7.5 g, 73%).

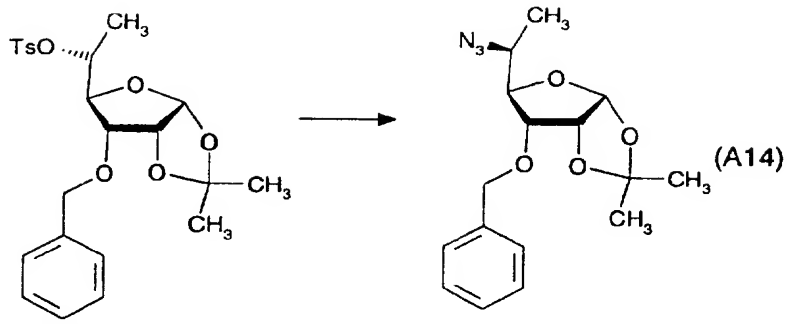
^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.60, 1.37 (2s, 6H, CH_3); 1.23 (d, J = 6 Hz, 3H, H-C(6'))
MS (CI): 312 ($M+NH_4^+$)



(A13)

A solution of compound **A12** (12,5 g, 42.6 mmol) in pyridine (125 ml) at 0°C is treated with toluene-4-sulfonyl-chloride (20.3 g, 106 mmol) and DMAP (520 g, 4.3 mmol). The reaction is slowly heated to 70°C and stirred for 3.0 h. The reaction mixture is poured into aqueous, saturated NH_4Cl solution, extracted with CH_2Cl_2 (3x), dried (Na_2SO_4) concentrated and purified by flash chromatography (silica, 25 - 35% EtOAc in hexane) to give compound **A13** (15,9 g, 84%)

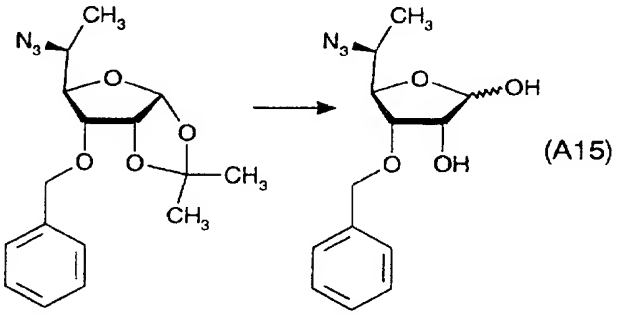
^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.34 (d, J = 6 Hz, 3H, H-C(6')); 1.32 (s, 3H, CH_3)
MS(CI): 448 (M^+), 357 ($M-PhCH_2$)



(A14)

A solution of compound **A13** (15.9 g, 36.0 mmol) in DMF (120 ml) is treated with NaN_3 (4.6 g, 71.2 mmol) and stirred at 80°C for 3.0 h. The reaction mixture is poured into Brine and extracted with EtOAc (3x). The combined organic layers are dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 20% EtOAc in hexane) to give compound **A14** (10.6 g, 93%).

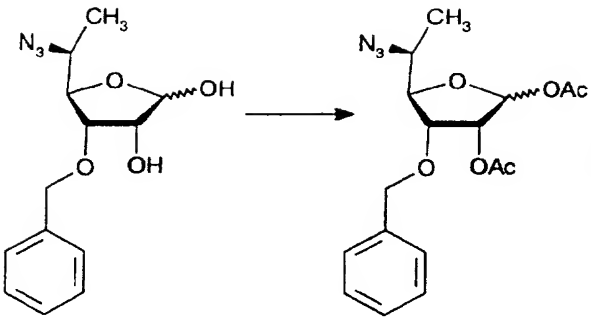
^1H NMR (500 MHz, $CDCl_3$): δ = 1.60 (s, 3H, CH_3); 1.44 (d, J = 7 Hz, 3H, H-C(6')); 1.38 (s, 3H, CH_3); MS(EI): 320 ($M+H^+$)



(A15)

A solution of compound **A14** (5.0 g, 15.7 mmol) in CH_2Cl_2 (25 ml) at 0°C is treated with H_2O (2.9 ml) and CF_3COOH (5.8 ml). The reaction mixture is stirred for 9.0 h at 25°C, cooled to 0°C and carefully treated with solid $NaHCO_3$. The reaction mixture is stirred for 0.3 h diluted with CH_2Cl_2 and washed with CH_2Cl_2. The aqueous phase is extracted with CH_2Cl_2 (2x), the combined organic layers are dried (Na_2SO_4) and concentrated to give compound **A15** (4.4 g, 100%). A small fraction is purified by flash chromatography (silica, 3% MeOH in CH_2Cl_2) for analysis.

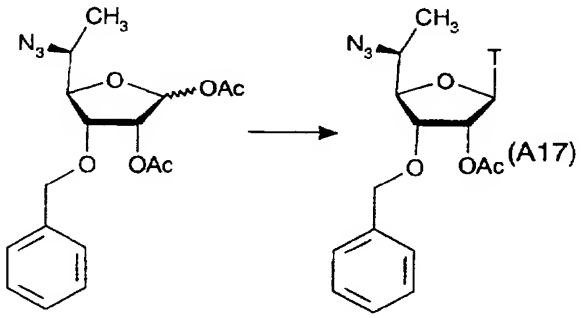
R_f = 0.35, 0.27 (silica, 4% MeOH in CH_2Cl_2)



(A16)

A solution of crude compound **A15** (4.4 g, 15.8 mmol) in pyridine (50 ml) is treated with Ac_2O (8.1 g, 79.0 mmol) and DMAP (0.2 g, 1.6 mmol). The reaction mixture is stirred for 0.5 h at 25°C, poured into saturated, aqueous solution of NH_4Cl and extracted with CH_2Cl_2 (3x). The combined organic layers are dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 15 - 20% EtOAc in hexane) to give compound **A16** (4.7 g, 92%, mixture of anomers (3.5:1 by ^1H NMR))

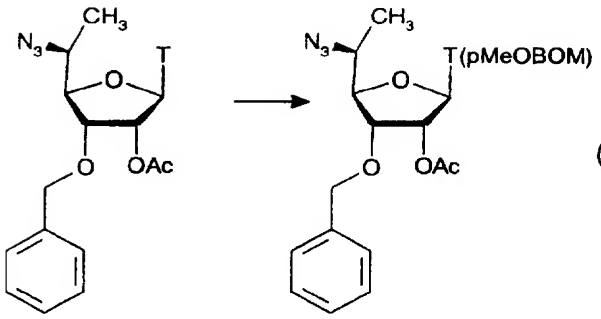
^1H NMR of less polar, major anomer (500 MHz, $CDCl_3$): δ = 2.14, 2.11 (2s, 6H, OAc); 1.41 (d, J = 7 Hz, 3H, H-C(6')); MS(FD): 363 (M)



(A17)

A solution of compound **A16** (4.1 g, 12.0 mmol) and thymine (2.1 g, 16.8 mmol) in CH_3CN (40 ml) is treated with N,O-bis(trimethylsilyl)acetamid (5.8 g, 28.4 mmol) and stirred for 0.5 h at 50°C. Trimethylsilyltrifluoromethane-sulfonate (5.7 g, 25.8 mmol) is added to the reaction mixture and stirring is continued for 3.0 h at 50°C. The reaction mixture is cooled to 25°C, poured into saturated, aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (3x). The combined organic layers are dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 50% EtOAc in hexane) to give compound **A17** (4.42 g, 80%).

^1H NMR (250 MHz, $CDCl_3$): δ = 2.15 (s, 3H, OAc); 1.95 (s, 3H, CH_3); 1.42 (d, 3H, H-C(6'))

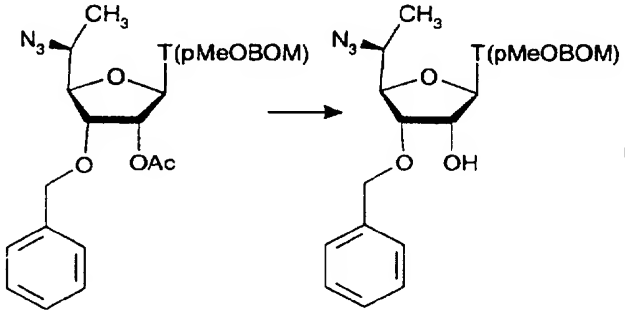


(A18)

A solution of compound **A17** (10.6 mg, 24.6 mmol) in DMF (70 ml) at 0°C is treated with DBU (7.5 g, 49.2 mmol) and a solution of p-methoxybenzyloxymethylchloride (8.3 g, 44.3 mmol) in DMF (30 ml). The reaction mixture is stirred for 2.0 h (0°C - 25°C), concentrated and purified by flash chromatography (30 - 50% EtOAc in hexane) to give compound **A18** (12.3 g, 87%).

R_f = 0.27 (silica, 33% EtOAc in hexane)

^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.79 (s, 3H, OCH_3); 2.15 (s, 3H, OAc); 1.95 (s, 3H, CH_3)

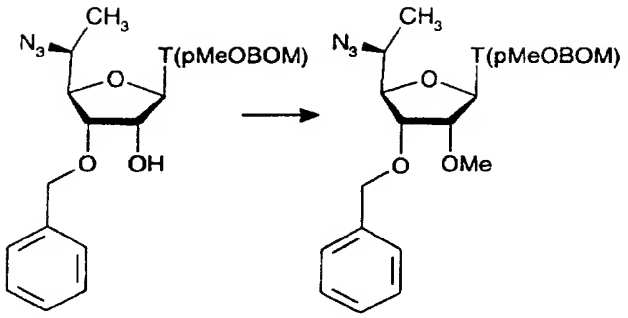


(A19)

A solution of compound **A18** (12.3 g, 21.3 mmol) in MeOH (120 ml) at 0°C is treated with NaOMe (4.6 g, 85.2 mmol) and stirred for 1.0 h at 0°C. The reaction mixture is poured into aqueous, saturated NH_4Cl-solution, extracted with CH_2Cl_2 (3x), dried (Na_2SO_4), adsorbed on Silica gel and purified by flash chromatography (50% EtOAc in hexane) to give compound **A19** (10.8 g, 94%)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.80 (s, 3H, OCH_3); 1.94 (d, J = 1 Hz, 3H, CH_3)

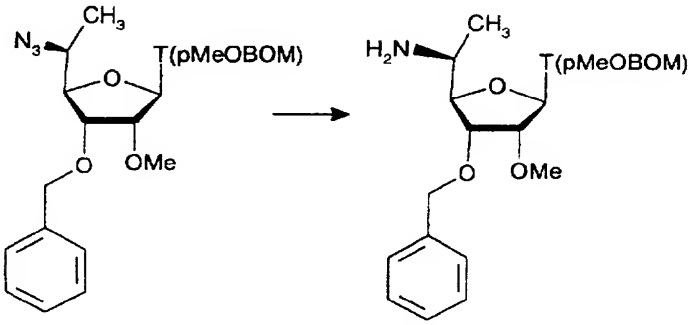
MS(CI): 555 ($M+NH_4^+$), 538 ($M+H^+$)



(A20)

To a solution of compound **A19** (10.3 g, 19.1 mmol) in THF (100 ml) at 0°C is added NaH (2.3 g, 57.3 mmol) and the reaction mixture is stirred for 0.5 h at 0°C. MeI is added to the reaction mixture and stirring is continued for 1.0 h at 0°C. The reaction mixture is poured into aqueous, saturated NH_4Cl-solution, extracted with CH_2Cl_2 (3x), the combined organic layers are dried (Na_2SO_4), concentrated and purified by flash chromatography (30% EtOAc in hexane) to give compound **A20** (10.8 g, 100%)

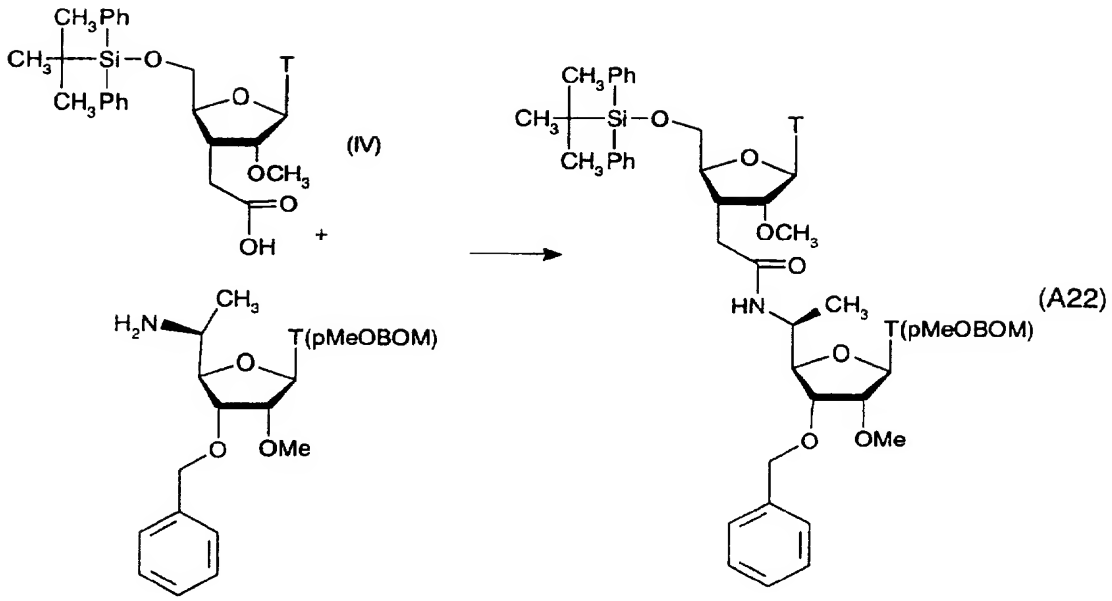
^1H NMR (500 MHz, $CDCl_3$): δ = 3.79 (s, 3H, $ArOCH_3$); MS(CI): 569 ($M+NH_4^+$), 552 ($M+H^+$)



(A21)

To a solution of compound **A20** (2.0 g, 3.63 mmol) in MeOH (3 ml) is added $SnCl_2 \cdot H_2O$ at 0°C and the reaction is stirred for 16.0 h (0 - 25°C). The reaction mixture is poured into saturated, aqueous $NaHCO_3$-solution and extracted with CH_2Cl_2 (3x). The combined organic layers are dried (Na_2SO_4), concentrated and purified by flash chromatography (5% MeOH in CH_2Cl_2) to give compound **A21** (1.4 g, 71%).

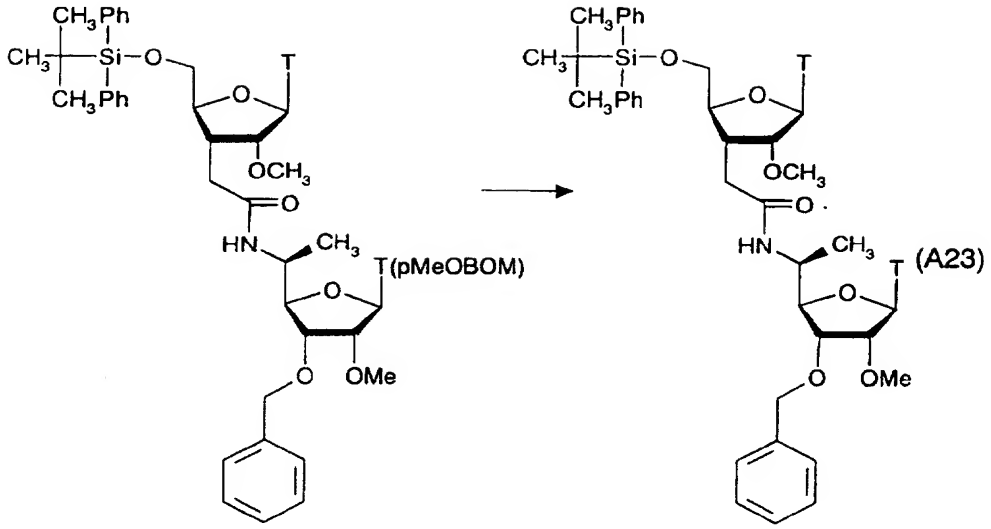
^1H NMR (500 MHz, $CDCl_3$): δ = 3.80 (s, 3H, $ArOCH_3$); 3.54 (s, 3H, OCH_3); MS(CI): 526 ($M+H^+$)



A solution of carboxylic acid **IV** (344 g, 0.62 mmol, dried over P_2O_5 on HV, 16.0 h) in CH_3CN (6 ml) is treated with Et_3N (70 mg, 0.685 mmol), O-(1-benztriazol-1-yl)-N,N,N,N-tetramethyluroniumtetrafluoroborat (220 mg, 0.685 mmol) and hydroxybenztriazol (42 mg, 0.312 mmol). The reaction mixture is stirred for 1.5 h. A solution of amine **A21** (327 mg, 0.632 mmol, dried over P_2O_5 on HV, 16.0 h) in CH_3CN (6 ml) and Et_3N (94 mg, 0.935 mmol) are added to the reaction mixture and stirring is continued for 0.5 h. The reaction mixture is poured into aqueous, saturated NaH_2PO_4-solution and concentrated. The aqueous phase is extracted with CH_2Cl_2 (3x), the combined organic layers are washed with aqueous, saturated NaH_2PO_4-solution, brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (1-2.5%MeOH in CH_2Cl_2) to give compound **A22** (539 mg, 90%).

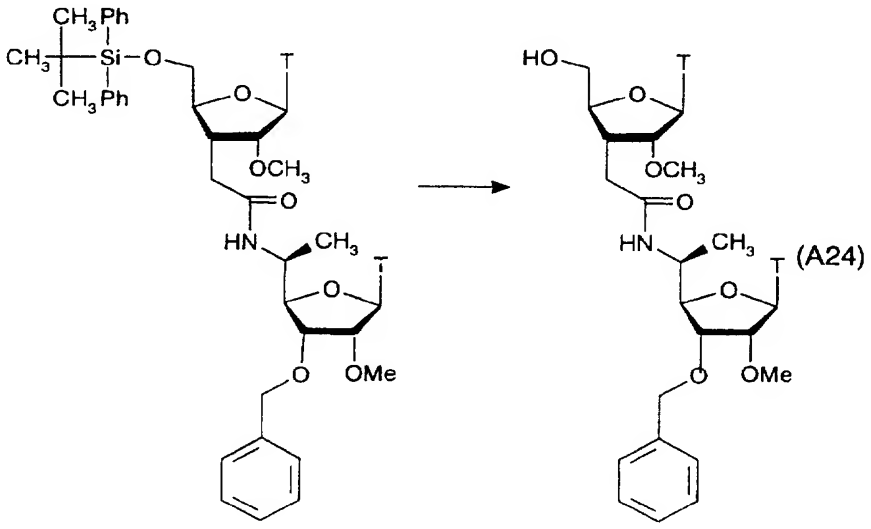
^1H NMR (500 MHz, $CDCl_3$): δ = 3.41 (2s, 6H, 2x OCH_3); 3.74 (3H, Ar-OCH_3);

MS(EI): 1058 ($M-H^+$)



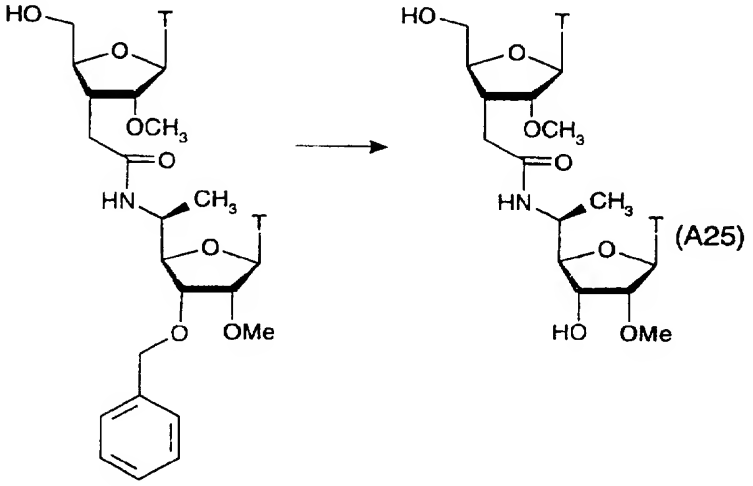
To a solution of compound **A22** (770 mg, 0.727 mmol) in CH_2Cl_2 (10 ml) and H_2O (1 ml) is added DDQ (430 mg, 1.89 mmol) in portions during 2 h and the reaction mixture is stirred for additional 0.5 h. The reaction mixture is filtered though celite, concentrated and purified by flash chromatography (5% MeOH in CH_2Cl_2). The chromatographed compound (mixture of product and hemiaminal) is dissolved in CH_2Cl_2 and rapidly stirred with saturated, aqueous Na_2CO_3 solution. The organic phase is separated from the aqueous phase, dried (Na_2SO_4) and concentrated to give compound **A23** (636 mg, 96%).

^{1}H NMR (500 MHz, $CDCl_3$): δ =3.40 and 3.43 (2s, 6H, 2x OCH_3); MS(CI): 909 (M^-)



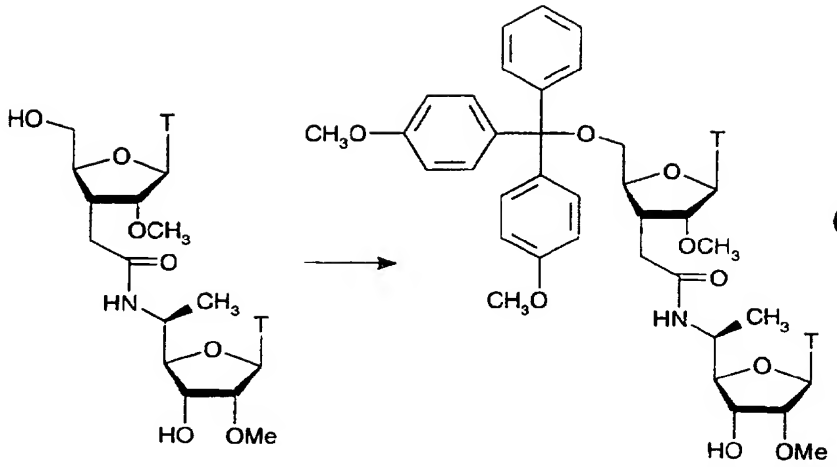
A solution of compound **A23** (630 mg, 0.693 mmol) in THF (8 ml) is treated with TBAF (1.04 ml of 1.0M solution in THF, 1.04 mmol) and stirred at 25°C for 1.5 h. The reaction is concentrated and purified by flash chromatography (5 - 7% MeOH in CH_2Cl_2) to give compound **A24** (393 mg, 85%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.56 and 3.40 (2s, 6H, 2x OCH_3);
MS(CI): 689 (M+NH4), 672 (M+H)



A solution of compound **A24** (383 mg, 0.57 mmol) degassed with argon, is treated with Pd/C (10%, 76 mg) and stirred under an H_2-atmosphere for 21 h. The reaction vessel is flushed with argon, filtered through celite, concentrated and purified by flash chromatography (15% MeOH in CH_2Cl_2) to give compound **A25** (290 mg, 88%).

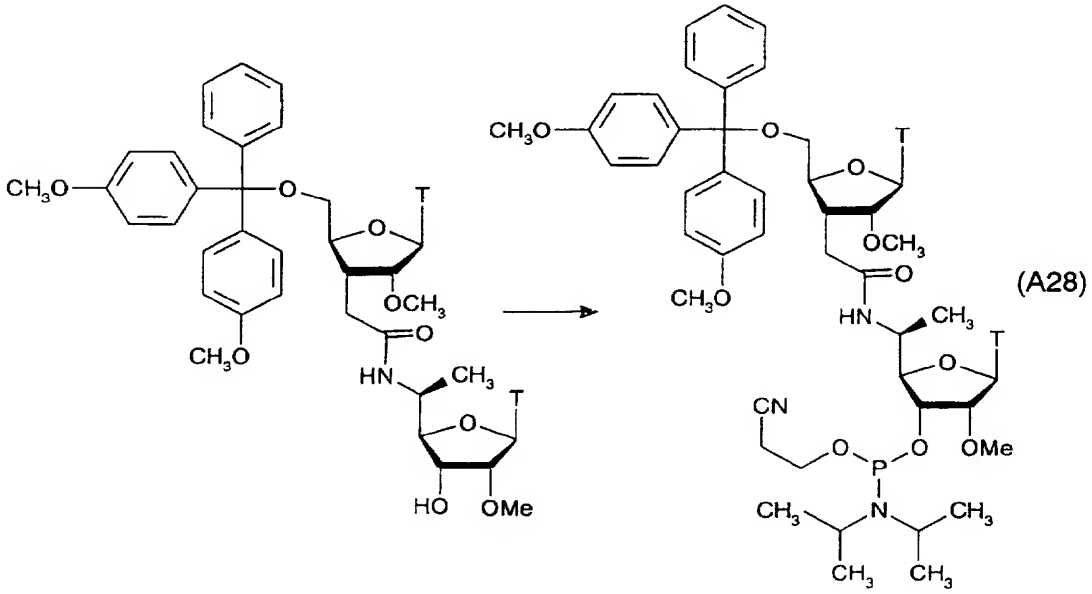
^{1}H NMR (250 MHz, CD_3OD): δ =3.47 and 3.45 (2s, 6H, 2x OCH_3); MS(EI): 580 (M-H)



(A27)

A solution of compound **A26** (288 mg, 0.496 mmol) in pyridine (3.5 ml) is treated with 4,4'-dimethoxytriphenylmethylchloride (406 mg, 1.20 mmol) and Et_3N (152 mg, 1.50 mmol) and stirred for 4 h at 25°C. The reaction mixture is poured into aqueous, saturated $NaHCO_3$-solution, extracted with CH_2Cl_2 (3x), dried (Na_2SO_4), concentrated, coevaporated with toluene (2x) and purified by flash chromatography (7% MeOH in CH_2Cl_2, 1% Et_3N) to give compound **A27** (380 mg, 87%).

^1H NMR (500 MHz, $CDCl_3$): δ = 3.52 and 3.49 (2s, 6H, 2x OCH_3); MS(EI): 882(M-H)



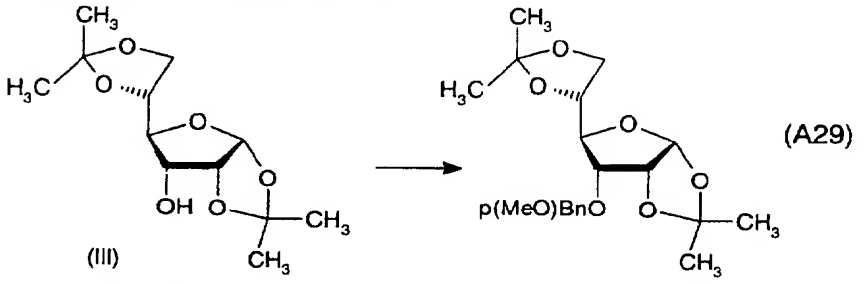
(A28)

Alcohol **A27** (300 mg, 0.339 mmol) and di-isopropylammonium tetrazolide (437 mg, 2.55 mmol) are dried for 12 h (HV), dissolved in CH_2Cl_2 (10 ml) and treated with cyanoethoxy-bis-

diisopropylamino-phosphine (460 mg, 1.53 mmol). The reaction mixture is stirred for 6 h at 25°C. The reaction is concentrated, dissolved in CH_2Cl_2 and precipitated in cold pentane. The mother liquor is concentrated and remaining product is precipitated. The precipitates are washed with pentane and purified by flash chromatography (3% MeOH, 1% Et_3N in CH_2Cl_2) to give phosphoramidite **A28** (350 mg, 95%,1:1 mixture of diastereomers).

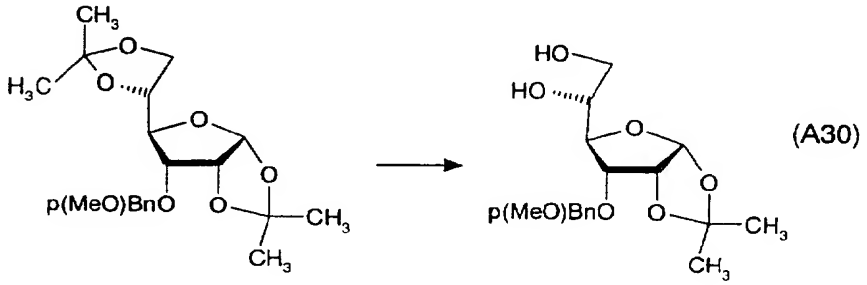
^{31}P NMR (101 MHz, $CDCl_3$): δ = 151.3, 150.2; MS(EI): 1082(M-H)

Example A3: Preparation of compound (A49)



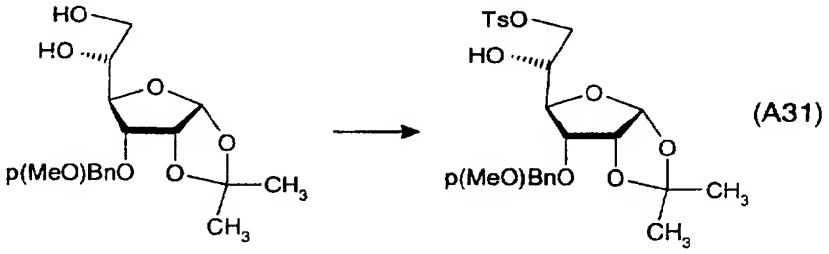
A solution of compound **III** (20 g, 0.077 mol) in THF (70 ml) is added to a suspension of NaH (3.69 g, 55%, 0.085 mol, washed with hexane) in THF (130 ml) at 0°C. The reaction is stirred for 1.0 h at 0°C and 0.5 h at 25°C. 4-methoxybenzylchloride (18 g, 0.1152 mol) and Bu_4NI (1.42 g, 3.8 mmol) are added to the reaction mixture and stirring is continued for 48 h at 25°C. The reaction mixture is poured into a saturated, aqueous solution of NH_4Cl and extracted with EtOAc (3x). The combined organic layers are washed with Brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 25-35% EtOAc in hexane) to give compound **A29** (15.24 g, 52%)

^1H NMR (500 MHz, $CDCl_3$): δ = 3.79 (3H, OCH_3); MS (EI): 379 (M-H)



Compound **A29** (15.0 g, 0.039 mol) is dissolved in AcOH/H_2O (9/1, 1105 ml) and stirred for 2.0 h at 40°C. The reaction mixture is concentrated coevaporated with toluene (2x). The material is dissolved in CH_2Cl_2, washed with aqueous $NaHCO_3$, dried with Na_2SO_4, concentrated and purified by flash chromatography (silica, 65% EtOAc in hexane) to give diol **A30** (11.9 g, 89%)

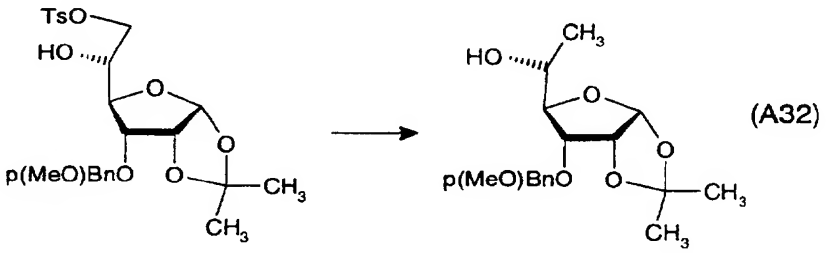
^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.82 (3H, OCH_3); MS(EI): 339 (M-H)



(A31)

A solution of compound **A30** (11.76 g, 34.6 mmol) in pyridine (100 ml) is treated with toluene-4-sulfonyl-chloride (9.23 g, 48 mmol) and DMAP (0.42 g, 3.5 mmol) at 0°C. The reaction is stirred for 4.0 h at 25°C, quenched with MeOH (11 ml), stirred for additional 0.3 h, concentrated, coevaporated with toluene (2x) and purified by flash chromatography to give compound **A31** (17.8 g, 89%)

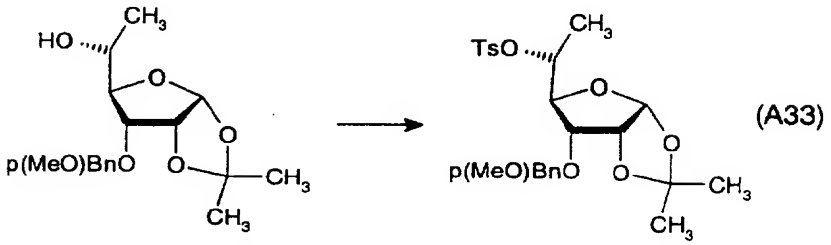
^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.82 (3H, OCH_3); MS(CI): 512 (M + NH_4)



(A32)

A solution of compound **A31** (15.15 g, 31 mmol) in DME (200 ml, degassed with Argon) is treated with NaI (13.8 g, 92.0 mmol), Bu_3SnH (13.53 g, 46.5 mmol) and AIBN (1.2 g, 6.2 mmol) and stirred for 1.0 h at 80°C. The reaction mixture is adsorbed onto silica gel, concentrated and purified by flash chromatography (silica, 30-50% EtOAc in hexane) to give compound **A32** (7.9 g, 79%)

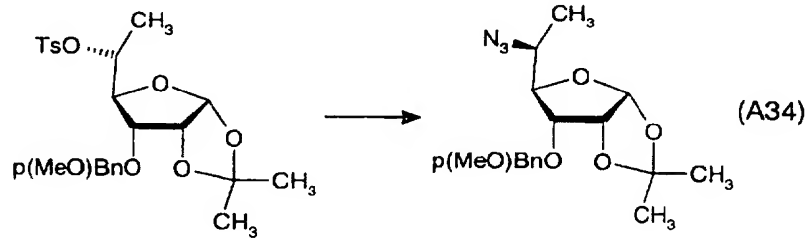
^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.81 (3H, OCH_3); MS (CI): 342 ($M+NH_4$)



(A33)

A solution of compound **A32** (7.9 g, 24 mmol) in pyridine (80 ml) at 0°C is treated with toluene-4-sulfonyl-chloride (11.62 g, 61 mmol) and DMAP (293 mg, 2.4 mmol). The reaction is heated to 70°C and stirred for 3.0 h. The reaction mixture is poured into aqueous, saturated NH_4Cl solution, extracted with CH_2Cl_2 (3x), dried (Na_2SO_4) concentrated and purified by flash chromatography (silica, 25 - 35% EtOAc in hexane) to give compound **A33** (9.14 g, 80%)

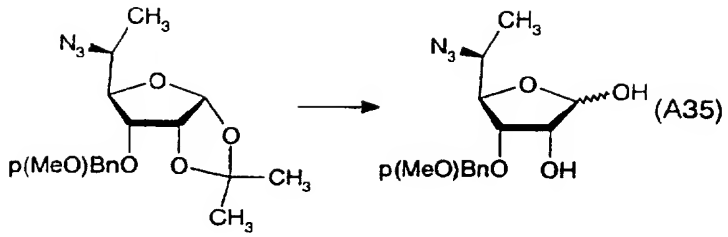
^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.82 (3H, OCH_3)



(A34)

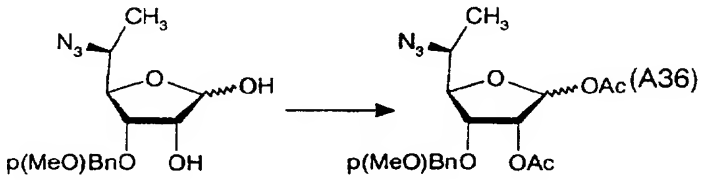
A solution of compound **A33** (8.94 g, 18.7 mmol) in DMF (90 ml) is treated with NaN_3 (3.65 g, 56 mmol) and stirred at 70°C for 16 h. The reaction mixture is poured into Brine and extracted with EtOAc (3x). The combined organic layers are dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 15-20% EtOAc in hexane) to give compound **A34** (6.0 g, 92%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.85 (3H, OCH_3); MS(CI): 367 ($M+NH_4$)



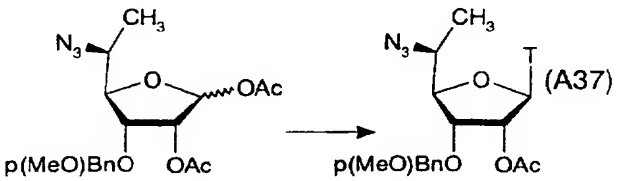
A solution of compound **A34** (6.0 g, 17.2 mmol) in 90% AcOH (90 ml) is stirred for 5 h at 80°C and 16 h at 25°C, cooled to 0°C and carefully treated with solid $NaHCO_3$. The reaction mixture is concentrated, coevaporated with toluene (2x) and purified by flash chromatography (silica, 35-50% EtOAc in hexane) to give **A35** (5.2 g, 98%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.83 (3H, OCH_3)



A solution of crude compound **A35** (3.3 g, 10.7 mmol) in pyridine (30 ml) is treated with Ac_2O (5.45 g, 53.0 mmol) and DMAP (0.13 g, 1.01 mmol). The reaction mixture is stirred for 0.5 h at 25°C, poured into saturated, aqueous solution of NH_4Cl and extracted with CH_2Cl_2 (3x). The combined organic layers are dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 25 - 35% EtOAc in hexane) to give compound **A36** (4.12 g, 98%, mixture of anomers (2.5:1 by ^{1}H NMR))

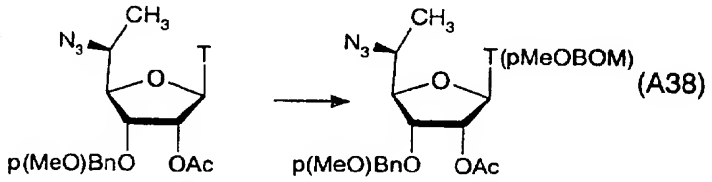
^{1}H NMR of less polar, major anomer (500 MHz, $CDCl_3$): δ = 3.81 (3H, OCH_3)



A solution of compound **A36** (4.12 g, 10.5 mmol) and thymine (1.72 g, 13.7 mmol) in CH_3CN (40 ml) is treated with N,O-bis(trimethylsilyl)acetamid (4.7 g, 23.1 mmol) and stirred for 0.5 h at 50°C. Trimethylsilyltrifluoromethane-sulfonate (4.67 g, 21 mmol) is added to the reaction

mixture and stirring is continued for 3.5 h at 50°C. The reaction mixture is cooled to 25°C, poured into saturated, aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (3x). The combined organic layers are dried (Na_2SO_4), concentrated and purified by flash chromatography (silica, 50% EtOAc in hexane) to give compound **A37** (4.13 g, 86%).

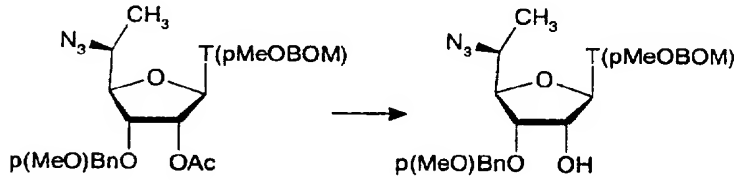
^1H NMR (250 MHz, $CDCl_3$): δ = 3.82 (3H, OCH_3); MS(EI): 458 (M-H)



(A38)

A solution of compound **A37** (4.13 g, 10 mmol) in DMF (30 ml) at 0°C is treated with DBU (2.74 g, 18.0 mmol) and a solution of p-methoxybenzyloxymethylchloride (3.02 g, 16.2 mmol) in DMF (10 ml). The reaction mixture is stirred for 2.0 h (0°C - 25°C), concentrated and purified by flash chromatography (50% EtOAc in hexane) to give compound **A38** (5.12 g, 93%)

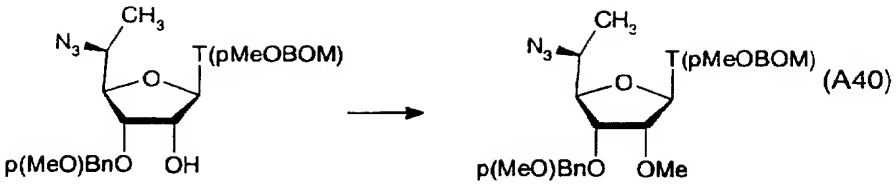
^1H NMR (250 MHz, $CDCl_3$): δ = 3.80 and 3.81 (2s, 6H, 2x OCH_3); MS(CI): 627 (M+NH4)



(A39)

A solution of compound **A38** (5.2 g, 8.4 mmol) in MeOH (50 ml) at 0°C is treated with NaOMe (1.82 g, 33.6 mmol) and stirred for 1.0 h at 25°C. The reaction mixture is poured into aqueous, saturated NH_4Cl-solution, extracted with CH_2Cl_2 (3x), dried (Na_2SO_4), adsorbed on silica gel and purified by flash chromatography (50% EtOAc in hexane) to give compound **A39** (4.47 g, 94%)

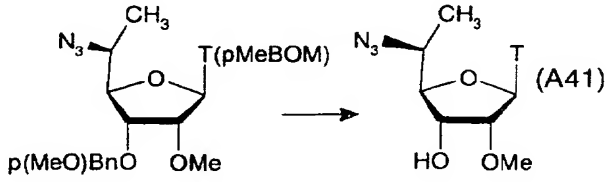
^1H NMR (500 MHz, $CDCl_3$): δ = 3.80 and 3.83 (2s, 6H, 2x OCH_3); MS(CI): 602 (M+Cl)



(A40)

To a solution of compound **A39** (3.0 g, 5.3 mmol) in THF (30 ml) at 0°C is added NaH (381 mg, 15.9 mmol) and the reaction mixture is stirred for 0.5 h at 0°C. MeI (7.52g, 53 mmol) is added to the reaction mixture and stirring is continued for 2.5 h at 0°C. The reaction mixture is poured into aqueous, saturated NH_4Cl-solution, extracted with CH_2Cl_2 (3x), the combined organic layers are dried (Na_2SO_4), concentrated and purified by flash chromatography (50% EtOAc in hexane) to give compound **A40** (3.04, 99%).

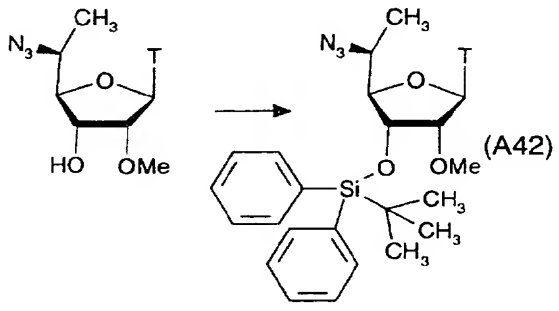
^1H NMR (500 MHz, $CDCl_3$): δ = 3.80 and 3.81 (2s, 6H, 2x OCH_3); MS(CI): 616 (M+Cl)



(A41)

To a solution of compound **A40** (3.4 g, 5.2 mmol) in CH_2Cl_2/H_2O (33 ml, 10:1) is added DDQ (4.93 g, 21.7 mmol) in portions during 1.5h. The reaction is stirred for an additional 1h at 25°C, filtered though Celite, concentrated and purified by flash chromatography (silica, 60-80% EtOAc in hexane) to give compound **A41** (1.25g, 77%).

^1H NMR (500 MHz, $CDCl_3$): δ = 3.60 (3H, OCH_3); MS(EI): 310 (M-H)

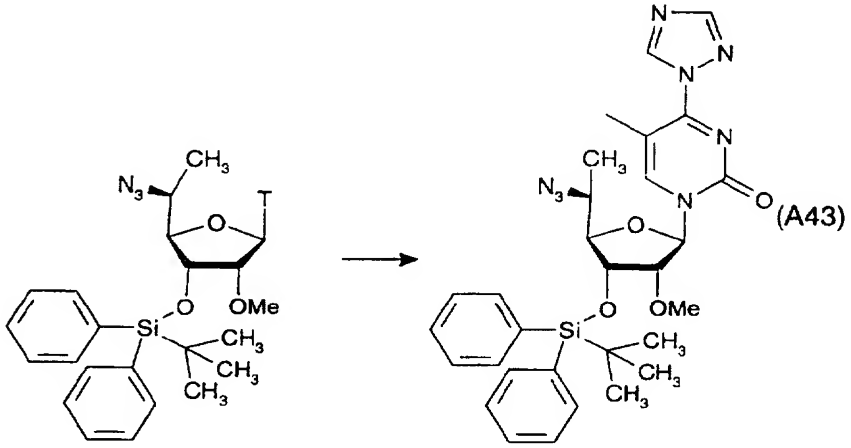


(A42)

A solution of compound **A41** (1.25 g, 4.0 mmol) and imidazol (554 mg, 8 mmol) in CH_2Cl_2 (20 ml) at 0°C is treated with t-butyldiphenylchlorosilane (1.76 g, 6.4 mmol) and stirred for 4h at 25°C. The reaction is quenched with MeOH (2 ml), stirred for 0.25 h, poured into

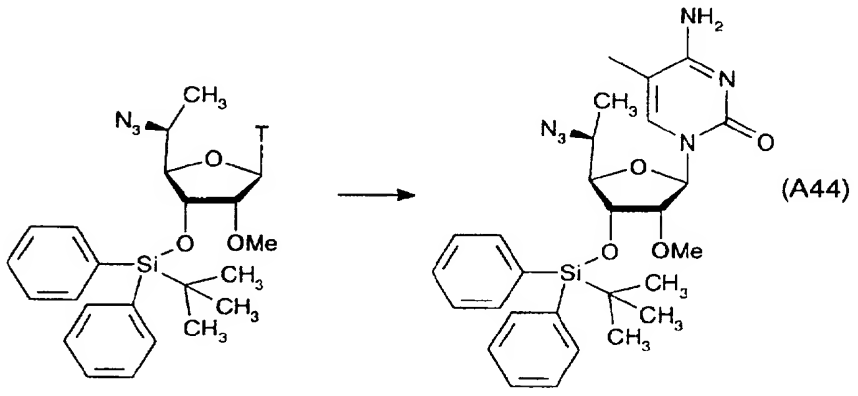
aqueous, saturated NH_4Cl-solution, extracted with CH_2Cl_2 (3x), the combined organic layers are washed with saturated, aqueous $NaHCO_3$ solution, dried (Na_2SO_4), concentrated and purified by flash chromatography (35% EtOAc in hexane) to give compound **A42** (1.85, 86%)

^1H NMR (500 MHz, $CDCl_3$): δ = 3.32 (3H, OCH_3)



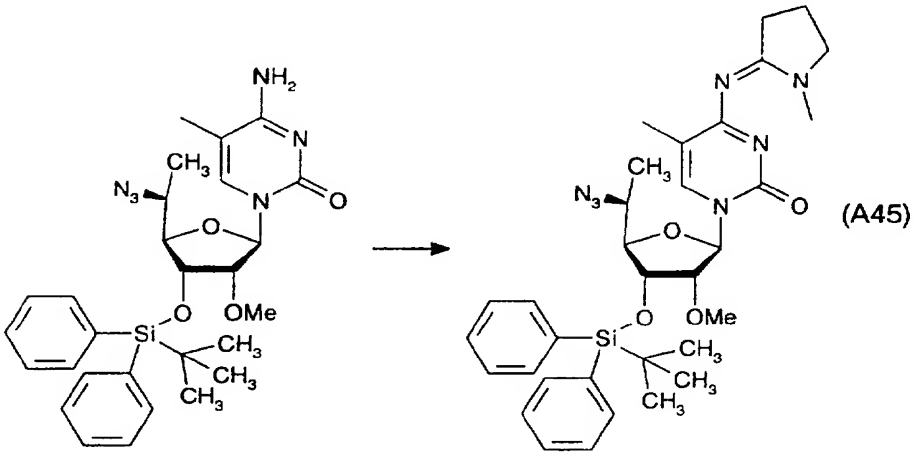
To a solution of compound **A42** (1.85 g, 3.45 mmol) in CH_3CN (20 ml) is added triazol (5.35 g, 77.5 mmol), Et_3N (8.2 g, 81 mmol) and the reaction is cooled to 0°C. $POCl_3$ (1.32 g, 8.6 mmol) is added slowly and the reaction is stirred for 0.5 h at 25°C. The reaction mixture is poured into saturated, aqueous $NaHCO_3$ solution, extracted with CH_2Cl_2 (3x), the combined organic layers are washed with brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (50% EtOAc in hexane) to give compound **A43** (1.91, 94%).

^1H NMR (500 MHz, $CDCl_3$): δ = 3.52 (3H, OCH_3)



To a solution of compound **A43** (1.91 g, 3.2 mmol) in dioxane (20 ml) is added NH_3 (10 ml, 25% in H_2O) and the reaction mixture is heated at 60°C for 0.5 h. The reaction mixture is concentrated, poured into saturated, aqueous $NaHCO_3$ solution, extracted with CH_2Cl_2 (3x), the combined organic layers are washed with brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (6% MeOH in CH_2Cl_2) to give compound **A44** (1.53, 86%).

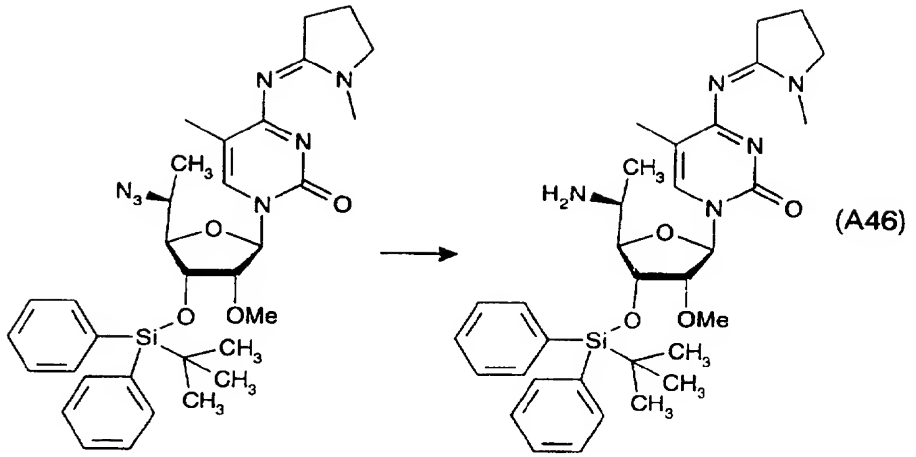
^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.45 (3H, OCH_3); MS(Cl): 583 (M+Cl)



(A45)

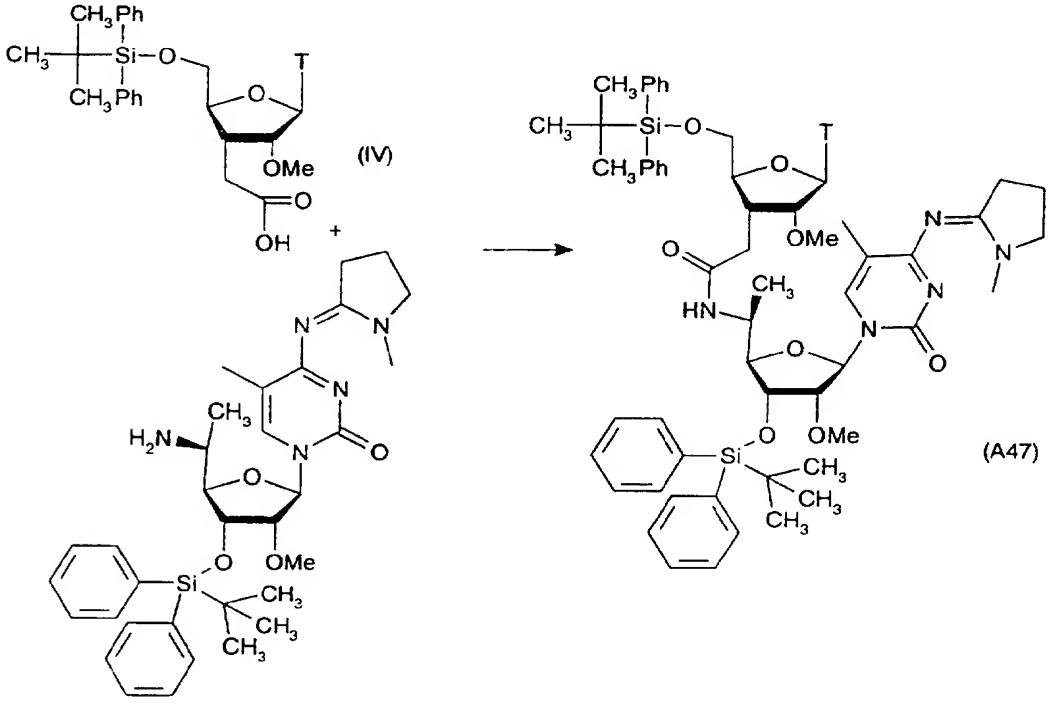
To a solution of compound **A44** (1.53 g, 2.8 mmol) in MeOH (15 ml) is added pyridine (1.11 g, 14 mmol) and N-methyl pyrolidone dimethylacetal (2.03 g, 14 mmol) and the reaction mixture is stirred at 25°C for 3 h. The reaction mixture is concentrated, coevaporated with toluene (2x) and purified by flash chromatography (4% MeOH in CH_2Cl_2) to give compound **A45** (1.41, 80%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.82 (s, 3H, N-CH_3); MS(EI): 630 (M+H)



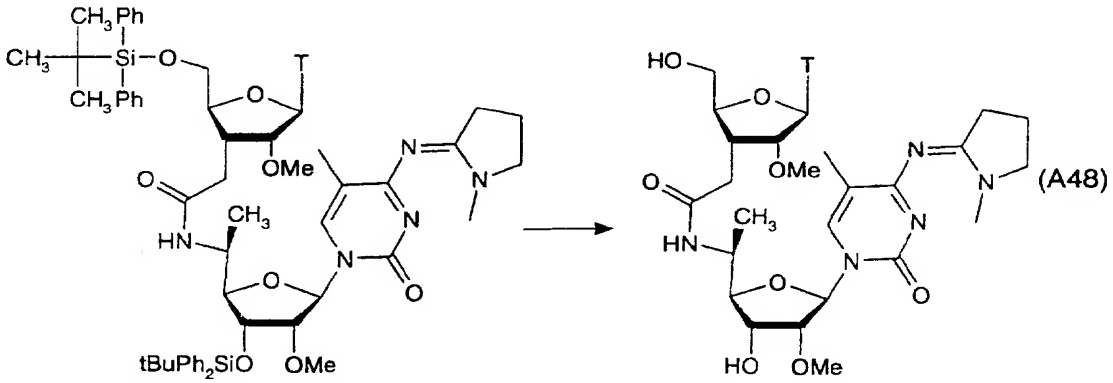
To a solution of compound **A45** (897.6 mg, 1.42 mmol) in MeOH (10 ml) is added $SnCl_2 \cdot 2H_2O$ (1.83 g, 8.31 mmol) in portions during 2h. The reaction is stirred for additional 3 h at 25°C. The reaction mixture is carefully quenched with saturated, aqueous $NaHCO_3$ solution, concentrated, redissolved in CH_2Cl_2. The organic layer is washed with brine, dried (Na_2SO_4), and concentrated to give crude compound **A46** (620 mg, 72%).

^1H NMR (500 MHz, $CDCl_3$): δ = 5.88 (1H,d,H-C(1'))



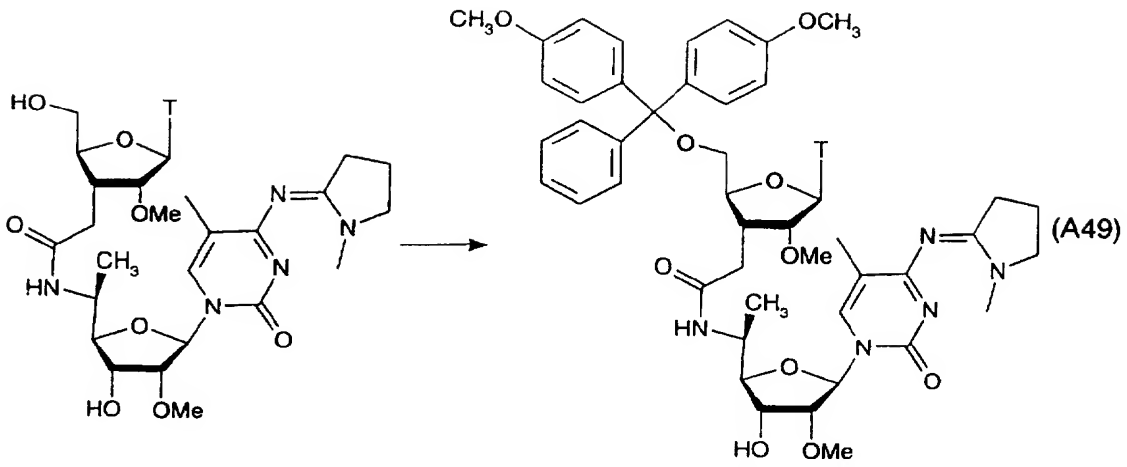
A solution of carboxylic acid **IV** (572 mg, 1.01 mmol, dried over P_2O_5 on HV, 16.0 h) in CH_3CN (8 ml) is treated with Et_3N (112 mg, 1.11 mmol), O-(1-benztriazol-1-yl)-N,N,N,N-tetramethyluroniumtetrafluoroborat (356 mg, 1.11 mmol) and hydroxybenztriazol (68 mg, 0.505 mmol). The reaction mixture is stirred for 2 h. A solution of amine **A46** (610 mg, 1.01 mmol, dried over P_2O_5 on HV, 16.0 h) in CH_3CN (5 ml) and Et_3N (153 mg, 1.51 mmol) are added to the reaction mixture and stirring is continued for 17 h. The reaction mixture is poured into aqueous, saturated NaH_2PO_4-solution and concentrated. The aqueous phase is extracted with CH_2Cl_2 (3x), the combined organic layers are washed with aqueous, saturated NaH_2PO_4-solution, brine, dried (Na_2SO_4), concentrated and purified by flash chromatography to give compound **A47** (652 mg, 80 %).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.19, 3.12, 3.05 (3s, 9H, 2x OCH_3, NCH_3); MS(EI): 1036 ($M-H^+$)



A solution of compound **A47** (650 mg, 0.59 mmol) in THF (10 ml) is treated with TBAF (1.34 ml of 1.0M solution in THF, 1.34 mmol) and stirred at 25°C for 4.5 h. The reaction is concentrated and purified by flash chromatography (5 - 20% MeOH in CH_2Cl_2) to give compound **A48** (341 mg, 88%).

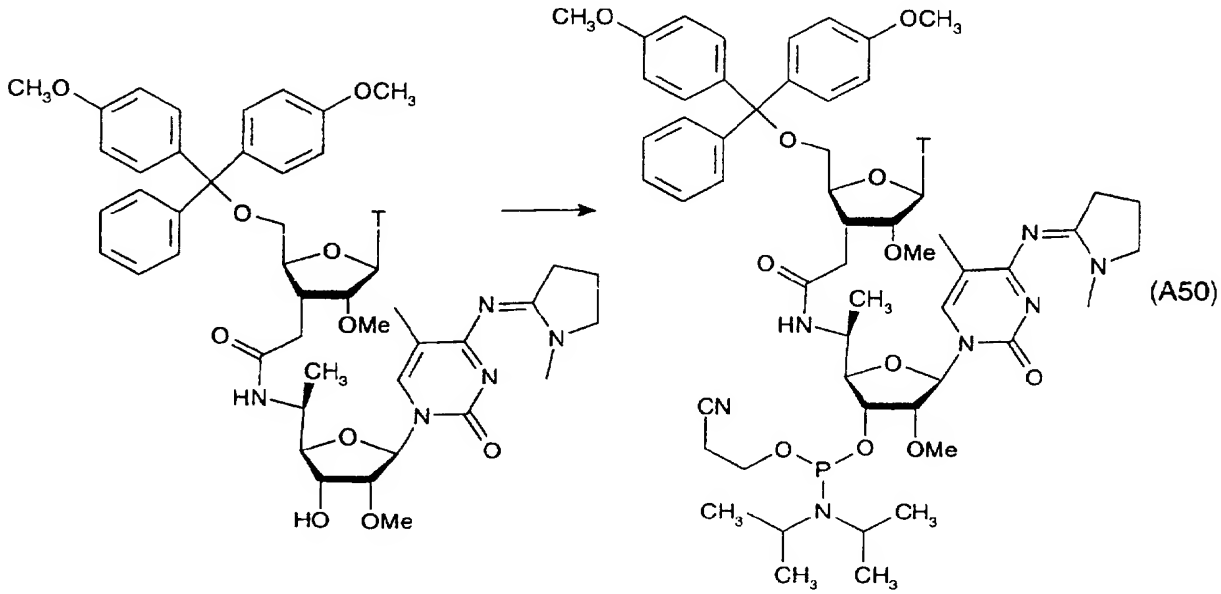
^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.18 (d, 3H, CH_3); MS(CI): 662 ($M+H^+$)



A solution of compound **A48** (335 mg, 0.506 mmol) in pyridine (10 ml) is treated with 4,4'-dimethoxytriphenylmethylchloride (265 mg, 0.76 mmol) and stirred for 22 h at 25°C. The reaction mixture is poured into aqueous, saturated $NaHCO_3$-solution, extracted with CH_2Cl_2 (3x), the organic layers are washed with brine, dried (Na_2SO_4), concentrated, coevaporated with toluene (3x) and purified by flash chromatography (10-20% MeOH in EtOAc, 1% Et_3N) to give compound **A49** (345 mg, 71%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.77 (2s, 6H, 2x $ArOCH_3$); 1.13 (d, 3H, CH_3)

MS(EI): 962 ($M-H^+$)

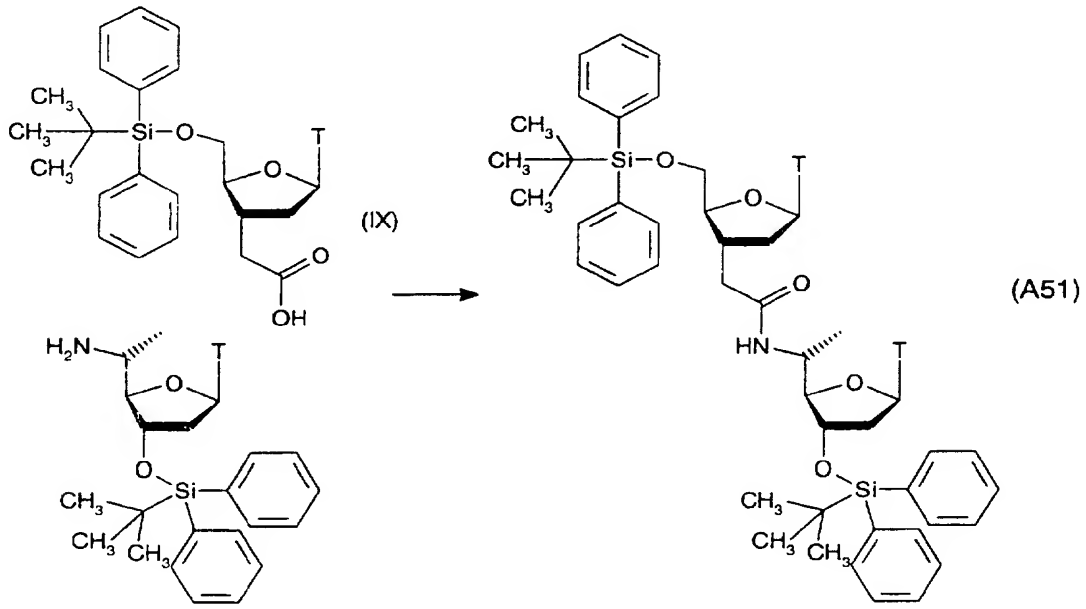


Alcohol **A49** (338 mg, 0.355 mmol), dissolved in CH_2Cl_2 (5ml), is added to a solution of di-isopropylammonium tetrazolide (67 mg, 0.0.391 mmol) and cyanoethoxy-bis-diisopropyl-

amino-phosphine (235 mg, 0.78 mmol) in CH_2Cl_2 (10 ml) at 25°C. The reaction mixture is stirred at RT for 2 h and is then poured into aqueous, saturated $NaHCO_3$-solution, extracted with CH_2Cl_2 (3x), the organic layers are washed with brine, dried (Na_2SO_4), concentrated, and purified by flash chromatography (2-10 % MeOH in EtOAc, 1% Et_3N) to give compound **A50** (366 mg, 88 %).

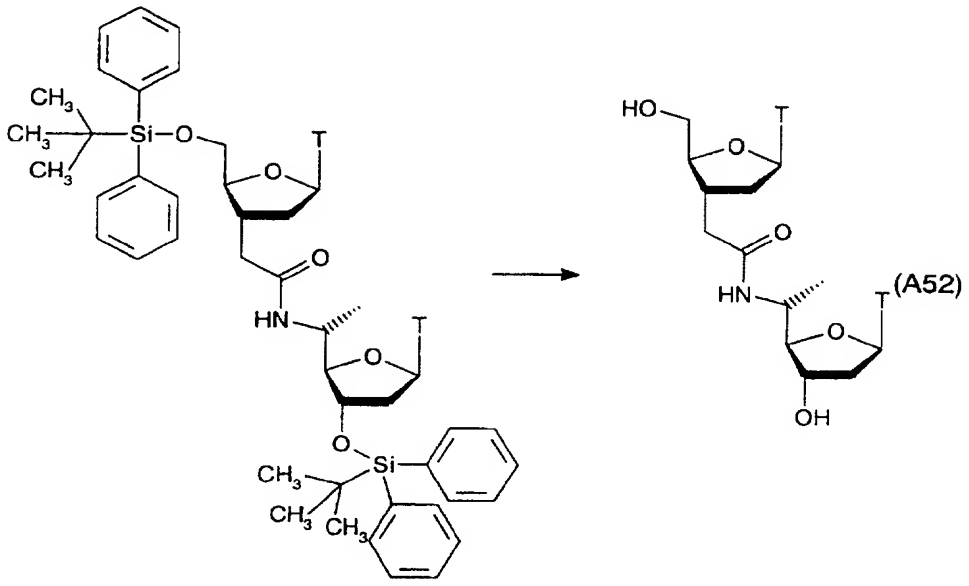
^{31}P NMR (101 MHz, $CDCl_3$): δ = 151.7, 150.8 (1:1 mixture of diastereomers).

Example A4: Preparation of compound (A54)



A solution of carboxylic acid **IX** (636 mg, 1.21 mmol, dried over P_2O_5 on HV, 16.0 h) in CH_3CN (6 ml) is treated with Et_3N (138 mg, 1.33 mmol), O-(1-benztriazol-1-yl)-N,N,N,N-tetramethyluroniumtetrafluoroborat (430 mg, 1.33 mmol) and hydroxybenztriazol (82 mg, 0.61 mmol). The reaction mixture is stirred for 1 h. A solution of amine **A4** (600 mg, 1.21 mmol, dried over P_2O_5 on HV, 16.0 h) in CH_3CN (4 ml) and Et_3N (138 mg, 1.33 mmol) are added to the reaction mixture and stirring is continued for 3 h. The reaction mixture is poured into aqueous, saturated NaH_2PO_4-solution and concentrated. The aqueous phase is extracted with CH_2Cl_2 (3x), the combined organic layers are washed with aqueous, saturated NaH_2PO_4-solution, brine, dried (Na_2SO_4), concentrated and purified by flash chromatography (2-5% MeOH in CH_2Cl_2) to give compound **A51** (1.14 g, 94 %).

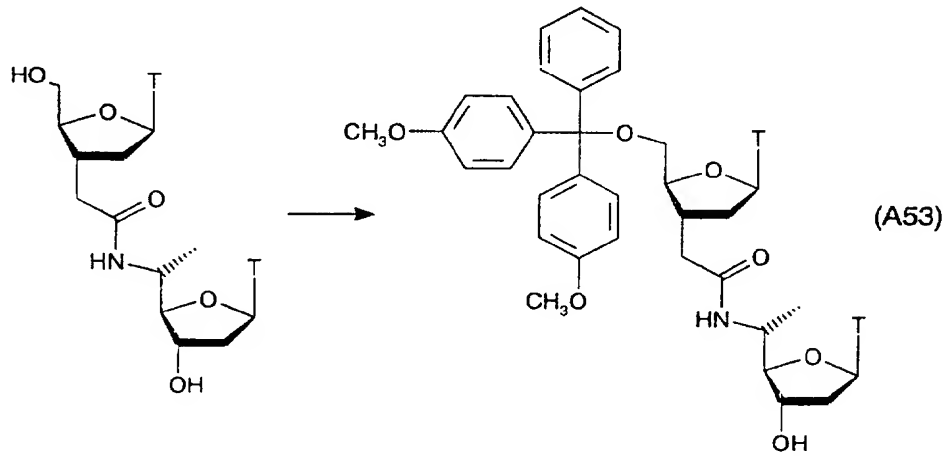
^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.31, 6.18 (2dd, 2H, 2x H-C(1')); MS(EI): 996 (M-H)



(A52)

A solution of compound **A51** (700 mg, 0.70 mmol) in THF (5 ml) is treated with TBAF (1.54 ml of 1.0M solution in THF, 1.54 mmol) and stirred at 25°C for 4 h. The reaction is concentrated and purified by flash chromatography (12 - 15% MeOH in CH_2Cl_2) to give compound **A52** (316 mg, 86%).

^{1}H NMR (400 MHz, CD_3OD): δ = 6.21, 6.07 (2dd, 2H, 2x H-C(1')); MS(EI): 520 (M-H)

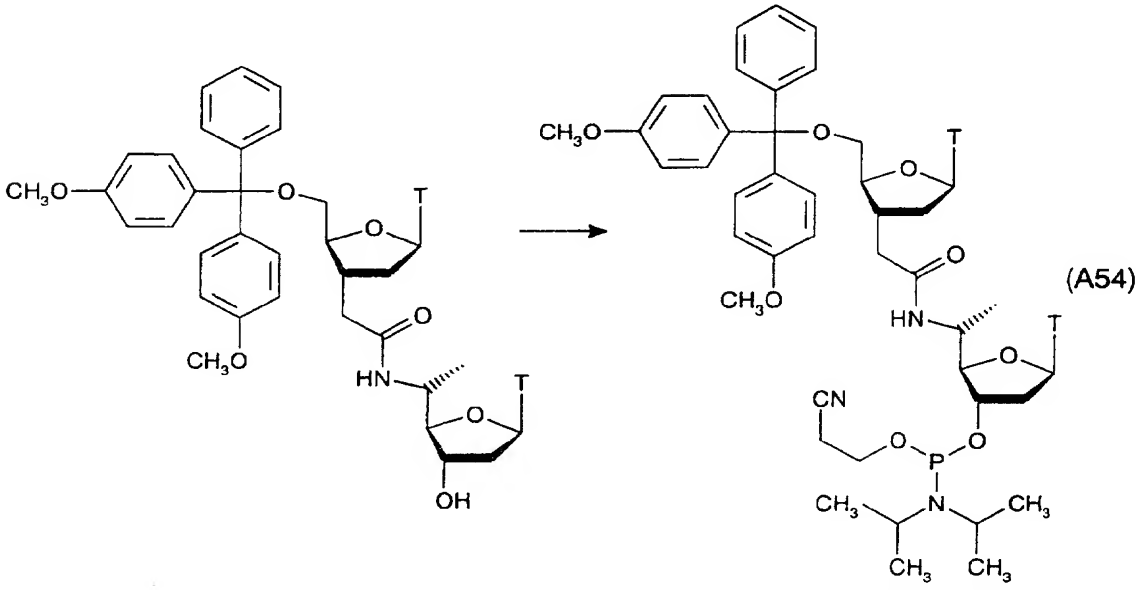


(A53)

A solution of compound **52** (290 mg, 0.56 mmol) in pyridine (3 ml) is treated with 4,4'-dimethoxytriphenymethylchloride (568 mg, 1.68 mmol) in portioned and stirred for 24 h at

25°C. The reaction mixture is poured into aqueous, saturated $NaHCO_3$-solution, extracted with CH_2Cl_2 (3x), the organic layers are washed with brine, dried (Na_2SO_4), concentrated, coevaporated with toluene (3x) and purified by flash chromatography (5-10% MeOH in CH_2Cl_2 , 1% Et_3N) to give compound **53** (328 mg, 71 %).

^1H NMR (250 MHz, CD_3OD): δ = 6.25 (m, 1H, H-C(1')); MS(EI): 822 (M-H)



(A54)

Alcohol **A53** (315 mg, 0.38 mmol), dissolved in CH_2Cl_2 (2ml), is added to a solution of di-isopropylammonium tetrazolide (44 mg, 0.256 mmol) and cyanoethoxy-bis-diisopropylamino-phosphine (172 mg, 0.0.573 mmol) in CH_2Cl_2 (2 ml) at 25°C. The reaction mixture is stirred for 5 h, poured into aqueous, saturated $NaHCO_3$-solution, extracted with CH_2Cl_2 (3x), the organic layers are washed with brine, dried (Na_2SO_4), concentrated, and purified by flash chromatography (1-10 % MeOH in EtOAc, 1% Et_3N) to give compound **A54** (365 mg, 93 %).

^{31}P NMR (101 MHz, $CDCl_3$): δ = 149.8, 148.5 (2 diastereomers); MS(EI): 1023 (M-H)

B: Synthesis of oligonucleotides

Each oligonucleotide is prepared on an ABI 390 DNA synthesizer using standard phosphoramidite chemistry according to Gait, M.J., Oligonucleotide Synthesis: A Practical Approach, IRL Press, Oxford (1984) but with prolonged coupling times (10 min). Dimethoxytrityl oligonucleotides are purified by reverse phase HPLC (column: Nucleosil RPC_{18}, 10 µ, 10x 250 mm; eluent A: 50 mM triethylammonium acetate (TEAA), pH 7.0; eluent B: 50mM TEAA, pH 7.0 in 70 % acetonitrile; elution with gradient from 15 % to 45 % B in 45 min). After purification by HPLC the oligodeoxynucleotides are controlled by capillary gel electrophoresis (concentration: 1 OD/ml, injection: 2 kV, 3 sec, separation: 9kV, capillary: effective length 30 cm, inner diameter 100 µm, polyacrylamide 10 % T, buffer: 100 mM H_3PO_4, 100 mM Tris, 2 mM EDTA, 7 M urea pH 8.8). The molecular weight of each oligodeoxynucleotide is checked by mass spectroscopy [MALDI-TOF: Pieles, U., Zürcher, W., Schär, M., Moser, H., Nucl. Acids Res. 21:3191 (1993)]. The oligodeoxynucleotide is desorbed using 2,4,6-trihydroxyacetophenone as a matrix (detection of negatively charged ions) with diammonium hydrogen citrate as additive (25mM final concentration).

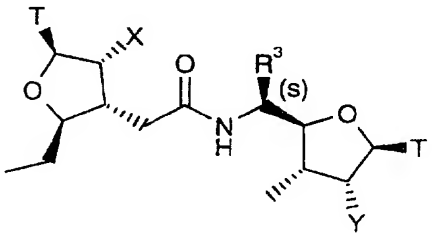
Oligonucleotides synthesized:

SEQ 1: 5'-GpCpGp**TsTpTsTpTsTpTsTpTsT**pGpCpG-3'

SEQ 2: 5' **TpTpTpTsTp**CpTpCpTpCpTpCpTpCpT-3'

p is an usual phosphordiester bond

TsT is



C: Properties of oligonucleotides

The thermal denaturation (T_m) of DNA/RNA hybrides is performed at 260 nm using a Gilford Response II Spectrophotometer (Ciba-Corning Diagnostics Corp., Oberlin, OH). Absorbance versus temperature profiles are measured at 4 µM of each strand in 10 mM

phosphate pH 7.0 (Na salts), 100 mM total [Na^+] (supplemented as NaCl), 0.1 mM EDTA. T_m's are obtained from fits of absorbance versus temperature curves to a two-state model with linear slope baselines [Freier, S.M., Albergo, D.D., Turner, D.H., Biopolymers 22:1107-1131 (1982)]. All values are averages of at least three experiments. The absolute experimental error of the T_m values is ± 0.5°C.

Binding to the complementary RNA strand (Δt_m / modification compared to wildtype)					
R^3	**X**	**Y**	**conf.**	**SEQ 1**	**SEQ 2**
CH_3	H	H	(S)	**+ 1.4**	**+ 1.0**
CH_3	H	H	(R)	**- 4.9**	**- 3.6**
H	H	H	-	- 0.9	+ 0.4

From these examples it is evident that a change in the configuration from (R) to (S) causes a dramatic increase in T_m. Surprisingly, Δt_m for the (S) configuration is even better than Δt_m in case of no substitution (R^3=H). This clearly shows that it is important to have a R^3 that is not hydrogen and that is bound in (S) configuration.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Novartis AG
(B) STREET: Schwarzwaldallee 215
(C) CITY: Basel
(E) COUNTRY: Switzerland
(F) POSTAL CODE (ZIP): 4058
(G) TELEPHONE: +41 61 696 11 11
(H) TELEFAX: + 41 61 696 79 76
(I) TELEX: 962 991

(ii) TITLE OF INVENTION: Modified oligonucleotides

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:4..5
(D) OTHER INFORMATION:/note= "modified backbone"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:6..7
(D) OTHER INFORMATION:/note= "modified backbone"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:8..9
(D) OTHER INFORMATION:/note= "modified backbone"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:10..11
(D) OTHER INFORMATION:/note= "modified backbone"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:12..13
(D) OTHER INFORMATION:/note= "modified backbone"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCGTTTTTTT TTTGCG 16

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:4..5
(D) OTHER INFORMATION:/note= "modified backbone"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

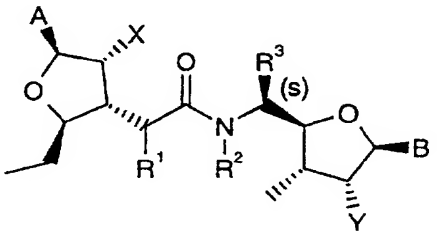
TTTTTCTCTC TCTCT 15

What is claimed is:

1. An oligonucleotide of formula 1

$$5'\text{-}(U)_n\text{-}3' \qquad (1),$$

in which U is an identical or different radical of a natural or a synthetic nucleoside, n is an integer from 2 to 200; and wherein the oligonucleotide of formula 1 comprises at least one structural unit of formula 2



(2)

wherein

R^1 is H, C_1-C_4alkyl or C_1-C_4alkoxy;

R^2 is H, C_1-C_4alkyl, phenyl, C_1-C_4alkyl-phenyl, C_3-C_9heteroaryl, C_1-C_4alkyl-C_3-C_9heteroaryl or an intercalator; wherein the aryl or heteroaryl is unsubstituted or substituted by OH, R^4, C_1-C_4alkoxy, -O-$(CH_2$-CH_2-$O)_m R^4$, $NR^4{}_2$ or NHR^4;

R^3 is C_1-C_4alkyl, unsubstituted or substituted by OH, $NR^4{}_2$ or NHR^4;

R^4 is H or C_1-C_4alkyl;

X and Y are independent of one another, H, OH, OR^4, O-C_1-C_4alkylNHR^4, O-C_1-C_4alkyl$NR^4{}_2$, -O-$(CH_2$-CH_2-$O)_m R^4$ or -O-CH_2-$C(OR^5)$H-CH_2-OR^6; or -O-CH_2-$C(OR^5)$H-CH_3;

R^5 is H, CH_3 or C_1-C_{10}alkyl;

R^6 is H, CH_3 or an OH-protecting group;

m is an integer from 1 to 4;

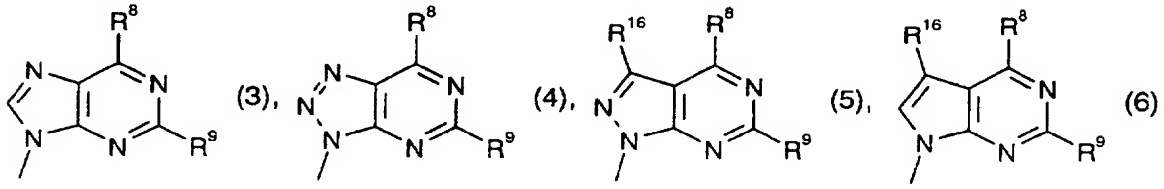
A and B are, independent of one another, a purine or pyrimidine radical or an analogue thereof;

with the proviso that if A and B are thymidine, R^1, R^2 and X are hydrogen and Y is methoxy, R^3 is not methyl.

2. The oligonucleotide of claim 1 wherein the intercalator is anthraquinone connected via a linker.

3. The oligonucleotide of claim 2 wherein the linker is a chain of 2 to 7 atoms selected from the group consisting of C, N and O.

4. The oligonucleotide according to claim 1 in which A and/or B as a purine radical or an analogue thereof is a radical of formula 3, 4, 5 or 6



in which

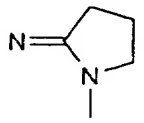
R^8 and R^9 independently of one another are H, OH, SH, NH_2, $NHNH_2$, NHOH, NHOalkyl having 1 to 12 C atoms, $-N=CH-N(C_1-C_{12}alkyl)_2$, F, Cl, Br, alkyl or hydroxyalkyl or aminoalkyl or alkoxy or alkylthio having 1 to 12 C atoms;

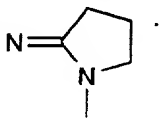
R^{16} is H, F, Cl, Br, $CONH_2$, alkyl, propinyl or hydroxyalkyl or aminoalkyl or alkoxy or alkylthio having 1 to 12 C atom.

5. The oligonucleotide according to claim 4, in which the primary amino contains 1 to 12 C atoms and the secondary amino 2 to 12 C atoms.

6. The oligonucleotide according to claim 4, in which the primary amino and secondary amino are radicals of the formula $R^{13}R^{14}N$ in which R^{13} and R^{14} are independently H, C_1-C_{20}alkyl, -aminoalkyl or -hydroxyalkyl; carboxyalkyl or carbalkoxyalkyl, where the carbalkoxy group contains 2 to 8 C atoms and the alkyl group contains 1 to 6, C atoms; C_2-C_{20}alkenyl; phenyl, mono- or di(C_1-C_4alkyl- or -alkoxy)phenyl, benzyl, mono- or di(C_1-C_4alkyl- or -alkoxy)benzyl; or 1,2-, 1,3- or 1,4-imidazolyl-C_1-C_6alkyl; or R^{13} and R^{14} together are tetra- or pentamethylene, 3-oxa-1,5-pentylene, $-CH_2-NR^{15}-CH_2CH_2-$ or $-CH_2CH_2-NR^{15}-CH_2CH_2-$, in which R^{15} is H or C_1-C_4alkyl; and the amino group in the aminoalkyl is unsubstituted or substituted by one or two C_1-C_4alkyl or -C_1-C_4hydroxyalkyl groups; and the hydroxyl group in hydroxyalkyl is unsubstituted or etherified with C_1-C_4alkyl.

7. The oligonucleotide according to claim 5, in which the primary amino and secondary amino are selected from the group consisting of methyl-, ethyl-, dimethyl-, diethyl-, allyl-, mono- or di(hydroxyeth-2-yl)-, phenyl-, benzyl-, acetyl-, isobutyryl-, benzoylamino, phenoxyacetylamino, 4-tert.-butylphenoxyacetylamino, $N=CH-N(CH_3)_2$, $N=CH-N(C_4H_9)_2$, and

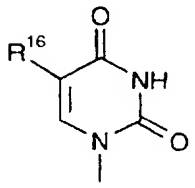
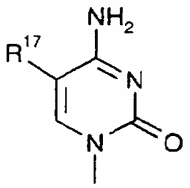


8. The oligonucleotide according to claim 4, in which R^8 and R^9, independent of one another, are H, F, Cl, Br, OH, SH, NH_2, NHOH, $NHNH_2$, methyl, methylamino, dimethylamino, benzoylamino, methoxy, ethoxy, methylthio, phenoxyacetylamino, 4-tert.-butylphenoxyacetylamino, $N=CH-N(CH_3)_2$, $N=CH-N(C_4H_9)_2$, and  .

9. The oligonucleotide according to claim 1, in which A or B are independent of one another a purine radical or a radical of a purine analogue from the series consisting of adenine, N-methyladenine, N-benzoyladenine, 2-methylthioadenine, 2-amino-6-chloropurine, 2-amino-6-methylthiopurine, 2-aminopurine, hypoxanthine, 2-aminoadenine, 2-hydroxypurine, guanine, N-isobutyrylguanine and .

10. The oligonucleotide according to claim 1, in which A or B are independent of one another a purine radical or a radical of a purine analogue from the series consisting of adenine, 2-aminoadenine, 2-aminopurine, guanine and hypoxanthine.

11. The oligonucleotide according to claim 1, in which A or B are independent of one another an analogous pyrimidine radical like a uracil, thymine or cytosine radical of the formulae 9 or 10

 (9),  (10),

in which R^{16} and R^{17} independently of one another are H, F, Cl, Br, alkyl, alkenyl, alkinyl, propargyl or hydroxyalkyl or aminoalkyl or alkoxy or alkylthio having 1 to 12 C atoms, phenyl, benzyl, primary amino having 1 to 20 C atoms or secondary amino having 2 to 30 C atoms, and the hydrogen atoms of the NH_2 group in formula 10 are unsubstituted or substituted by C_1-C_6alkyl, benzoyl or benzyl; and the dihydro derivatives of the radicals of formulae 9 and 10.

12. The oligonucleotide according to claim 10, in which R^{16} is H, F, Cl, Br, C_1-C_6alkyl, C_1-C_6alkenyl, C_1-C_6alkinyl, C_1-C_6hydroxyalkyl, C_1-C_6aminoalkyl, NHC_1-C_4alkyl, $N(C_1$-C_4alkyl$)_2$, propinyl.

13. The oligonucleotide according to claim 10, in which R^{16} is H, F, Cl, Br, methyl, ethyl, and propinyl.

14. The oligonucleotide according to claim 10, in which R^{17} is H, F, Cl, Br, C_1-C_6alkyl, C_1-C_6alkoxy, C_1-C_6hydroxyalkyl, C_1-C_6aminoalkyl, NH_2, NHC_1-C_4alkyl, $N(C_1$-C_4alkyl$)_2$, or propinyl.

15. The oligonucleotide according to claim 10, in which R^{17} is H, F, Cl, Br, methyl, ethyl, or propinyl.

16. The oligonucleotide according to claim 10, wherein R^{16} and R^{17} are H, methyl or propinyl.

17. The oligonucleotide according to claim 1, in which A and B are independent of one another as the radical of a pyrimidine analogue are derived from uracil, thymine, cytosine, 5-fluorouracil, 5-chlorouracil, 5-bromouracil, 5-methylcytosine, 5-propinyluracil, and 5-propinylcytosine.

18. The oligonucleotide according to claim 1, comprising at least one structural unit of formula 2 wherein

R^1 is H or C_1-C_4alkyl;

R^2 is H, C_1-C_4alkyl, phenyl, C_1-C_4alkyl-phenyl or C_3-C_9heteroaryl;

R^3 is C_1-C_4alkyl;

R^4 is methyl or ethyl;

X and Y are independent of one another, H, OH, OR^4, O-C_1-C_4alkylNHR^4, O-C_1-C_4alkyl$NR^4{}_2$, -O-$(CH_2$-CH_2-O$)_m R^4$;

R^5 is H or C_1-C_4alkyl.

19. The oligonucleotide according to claim 1, wherein

R^1 is H or methyl;

R^2 is H, methyl, ethyl or phenyl;

R^3 is methyl or ethyl;

R^4 is methyl;

X and Y are independent of one another, H, OH or OR^4; O-$CH_2CH_2NHR^4$, O-$CH_2CH_2NR^4{}_2$, O-$CH_2CH_2OR^4$;

R^5 is H or C_1-C_4alkyl.

20. The oligonucleotide according to claim 1, wherein

R^1 is H;

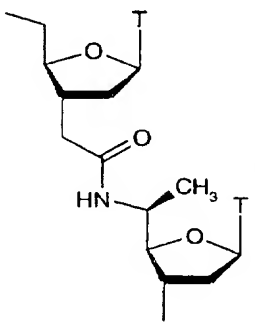
R^2 is H, methyl or phenyl;

R^3 methyl;

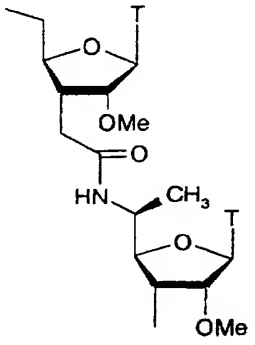
X and Y are independent of one another, H, O-CH_3, O-$CH_2CH_2OCH_3$, O-$CH_2CH_2NHCH_3$, O-$CH_2CH_2N(CH_3)_2$;

R^5 H or methyl.

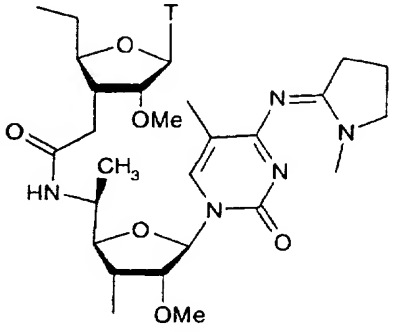
21. The oligonucleotide according to claim 1, wherein the structural unit of formula 2 is of formula B8, B28 or B49



B8;



B28;



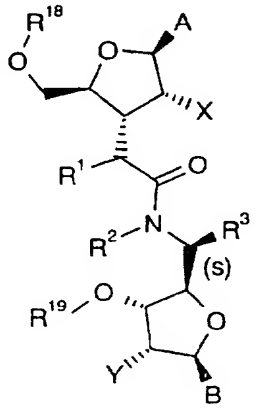
B49

22. The oligonucleotide of claim 1 wherein n is 2 to 100.

23. The oligonucleotide of claim 1 wherein n is 2 to 50.

24. The oligonucleotide of claim 1 wherein n is 2 to 20.

25. A nucleoside dimer of formula 12



(12)

wherein

R^1 is H, C_1-C_4alkyl or C_1-C_4alkoxy;

R^2 is H, C_1-C_4alkyl, C_1-C_4alkoxy, phenyl, C_1-C_4alkyl-phenyl, C_3-C_9heteroaryl, C_1-C_4alkyl-C_3-C_9heteroaryl or an intercalator; wherein the aryl or heteroaryl is unsubstituted or substituted by OH, R^4, C_1-C_4alkoxy, -O-$(CH_2$-CH_2-$O)_m R^4$, NR^4_2 or NHR^4;

R^3 is C_1-C_4alkyl, unsubstituted or substituted by OH, NR^4_2 or NHR^4;

R^4 is H or C_1-C_4alkyl;

X and Y are independent of one another, H, OH, OR^4, O-C_1-C_4alkylNHR^4, O-C_1-C_4alkylNR^4_2, -O-$(CH_2$-CH_2-$O)_m R^4$ or -O-CH_2-$C(OR^5)$H-CH_2-OR^6; or -O-CH_2-$C(OR^5)$H-CH_3;;

R^5 is H or C_1-C_{10}alkyl;

R^6 is H, CH_3 or an OH-protecting group;

m is an integer from 1 to 4;

A and B are, independent of one another, a purine or pyrimidine radical or an analogue thereof;

R^{18} and R^{19} are H, an OH-protecting group or a radical forming a phosphorus-containing nucleotide bridging group;

with the proviso that if A and B are thymidine, R_1, R^2 and X are hydrogen and Y is methoxy, R^3 is not methyl.

26. The nucleoside dimer according to claim 25 wherein R^{18} is H or an OH-protecting group and R^{19} is a phosphorus-containing, nucleotide-bridge-group-forming radical.

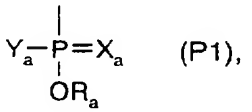
27. The nucleoside dimer according to claim 25 wherein the OH-protecting group is linear or branched C_1-C_8alkyl; C_7-C_{18}aralkyl; triphenylsilyl, alkyldiphenylsilyl, dialkylphenylsilyl or trialkylsilyl having 1 to 20 C atoms in the alkyl groups; -$(C_1$-C_8alkyl$)_2$Si-O-Si$(C_1$-C_8alkyl$)_2$-, C_2-C_{12}acyl, R^{12}-SO_2-, in which R^{12} is C_1-C_{12}alkyl, C_5- or C_6cycloalkyl, phenyl, benzyl, C_1-C_{12}alkylphenyl, C_1-C_{12}alkylbenzyl, or is C_1-C_{12}alkoxycarbonyl, phenoxycarbonyl, benzyl-oxycarbonyl, methylphenoxycarbonyl or methylbenzyloxycarbonyl which is unsubstituted or substituted by F, Cl, Br, C_1-C_4alkoxy, tri$(C_1$-C_4alkyl)silyl or C_1-C_4alkylsulfonyl, or 9-fluorenylmethoxycarbonyl.

28. The nucleoside dimer according to claim 27, wherein the OH-protecting group is linear or branched C_1-C_4alkyl, C_7-C_{18}aralkyl, trialkylsilyl having 1 to 12 C atoms in the alkyl groups; -$(CH_3)_2$Si-O-Si$(CH_3)_2$-; -$(i$-$C_3H_7)_2$Si-O-Si$(iC_3H_7)_2$-; C_2-C_8acyl; R^{12}-SO_2-, in which

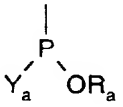
R^{12} is C_1-C_6alkyl; phenyl or benzyl unsubstituted or substituted with F, Cl or Br; C_1-C_4alkylphenyl, C_1-C_4alkylbenzyl; C_1-C_8alkoxycarbonyl; phenoxycarbonyl; benzyloxycarbonyl or 9-fluorenylmethoxycarbonyl.

29. The nucleoside dimer according to claim 27, wherein the OH-protecting group is methyl, ethyl, n- or i-propyl, n-, i- or t-butyl; benzyl, methylbenzyl, dimethylbenzyl, methoxybenzyl, dimethoxybenzyl, bromobenzyl; diphenylmethyl, di(methylphenyl)methyl, di(dimethylphenyl)methyl, di(methoxyphenyl)methyl, di(methoxyphenyl)(phenyl)methyl, trityl, tri(methylphenyl)ethyl, tri(dimethylphenyl)methyl, tri(methoxyphenyl)methyl, tri(dimethoxyphenyl)methyl; trimethylsilyl, triethylsilyl, tri-n-propylsilyl, i-propyldimethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, n-octyldimethylsilyl, (1,1,2,2-tetramethylethyl)dimethylsilyl, -$(CH_3)_2$Si-O-Si$(CH_3)_2$-, -$(i\text{-}C_3H_7)_2$Si-O-Si$(iC_3H_7)_2$-; acetyl, propanoyl, butanoyl, pentanoyl, hexanoyl, benzoyl, methylbenzoyl, methoxybenzoyl, chlorobenzoyl or bromobenzoyl; methyl-, ethyl-, propyl-, butyl-, phenyl-, benzyl-, p-bromo-, p-methoxy- or p-methylphenylsulfonyl; methoxy-, ethoxy-, n- or i-propoxy- or n-, i- or t-butoxycarbonyl, or phenoxycarbonyl, benzyloxycarbonyl, methyl- or methoxy- or chlorophenoxycarbonyl or -benzyloxycarbonyl or 9-fluorenylmethoxycarbonyl.

30. A nucleoside dimer according to claim 25 wherein the phosphorus-containing, nucleotide-bridge-group-forming radical may correspond to formula P1 or P2



(P1),



(P2)

wherein

Y_a is hydrogen, C_1-C_{12}alkyl, C_6-C_{12}aryl, C_7-C_{20}aralkyl, C_7-C_{20}alkaryl, -OR_b, -SR_b, secondary amino, O^-M^+ or S^-M^+;

X_a is oxygen or sulfur;

R_a is hydrogen, M^+, C_1-C_{12}alkyl, C_2-C_{12}alkenyl or C_6-C_{12}aryl, or the group R_aO- is N-heteroaryl-N-yl having 5 ring members and from 1 to 3 nitrogen atoms;

R_b is hydrogen, C_1-C_{12}alkyl or C_6-C_{12}aryl; and

M^+ is Na^+, K^+, Li^+, NH_4^+ or primary, secondary, tertiary or quaternary ammonium;

alkyl, aryl, aralkyl and alkaryl in Y_a, R_a and R_b being unsubstituted or substituted by alkoxy, alkylthio, halogen, -CN, -NO_2, phenyl, nitrophenyl or halophenyl.

31. A nucleotide dimer according to claim 30 wherein R_a is β-cyanoethyl and Y_a is di(iso-propyl)amino.

32. A nucleoside dimer according to claim 25, wherein

R^1 is H or C_1-C_4alkyl;

R^2 is H, C_1-C_4alkyl, phenyl, C_1-C_4alkyl-phenyl or C_3-C_9heteroaryl;

R^3 is C_1-C_4alkyl;

R^4 is methyl or ethyl;

X and Y are independent of one another, H, OH, OR^4, $-O-(CH_2-CH_2-O)_mR^4$;

R^5 is H or C_1-C_4alkyl.

33. A nucleoside dimer according to claim 25, wherein

R^1 is H or methyl;

R^2 is H, methyl, ethyl or phenyl;

R^3 is methyl or ethyl;

X and Y are independent of one another, H, OH or OR^4; $O-CH_2CH_2NHR^4$, $O-CH_2CH_2N(CH_3)_2$, $O-CH_2CH_2OCH_3$;

R^5 is H or C_1-C_4alkyl.

34. A nucleoside dimer according to claim 25, wherein

R^1 is H;

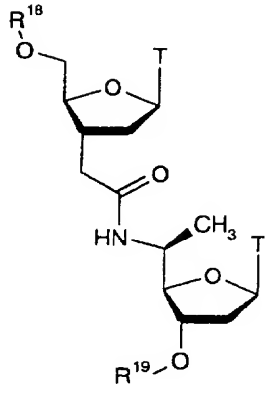
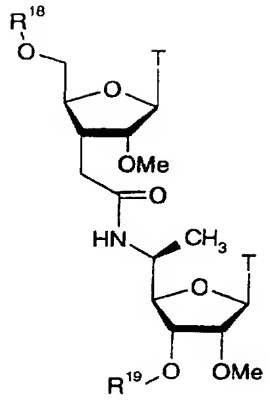
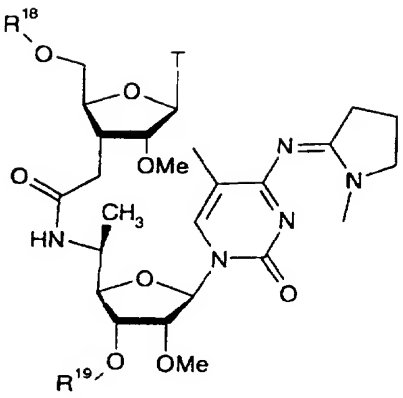
R^2 is H, methyl or phenyl;

R^3 methyl;

R^5 is H or methyl;

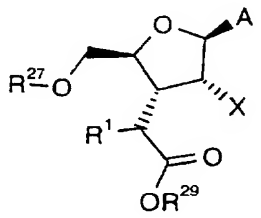
X and Y are independent of one another, H, $O-CH_3$, $O-CH_2CH_2OCH_3$, $O-CH_2CH_2NHCH_3$, $O-CH_2CH_2N(CH_3)_2$;

35. A nucleoside dimer according to claim 25, selected from the group consisting of compounds of formula C8, C28 and C49


C8;

C28,

C49

36. A process for the preparation of a nucleoside dimer according to claim 25 which comprises:

a compound of the formula 14


(14)

wherein

R^1 is H or C_1-C_4alkyl;

X is H, OH, OR^4, O-C_1-C_4alkylNHR^4, O-C_1-C_4alkylN$R^4{}_2$, -O-$(CH_2$-CH_2-$O)_mR^4$ or -O-CH_2-$C(OR^5)$H-CH_2-OR^6;

R^4 is H or C_1-C_4alkyl;

R^5 is H or C_1-C_{10}alkyl;

R^6 is H, CH_3 or an OH-protecting group;

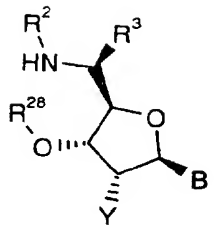
m is an integer from 1 to 4;

A is a purine or pyrimidine radical or an analogue thereof.

R^{27} is H or an OH-protecting group;

R^{29} is H or an ester activating group;

is reacted with a compound of the formula 15



(15)

wherein

R^2 is H, C_1-C_4alkyl, C_1-C_4alkoxy, phenyl, C_1-C_4alkyl-phenyl, C_3-C_9heteroaryl, C_1-C_4alkyl-C_3-C_9heteroaryl or an intercalator; wherein the aryl or heteroaryl is unsubstituted or substituted by OH, R^4, C_1-C_4alkoxy, -O-$(CH_2$-CH_2-$O)_mR^4$, NR^4_2 or NHR^4;

R^3 is C_1-C_4alkyl, unsubstituted or substituted by OH, NR^4_2 or NHR^4;

Y is H, OH, OR^4, O-C_1-C_4alkylNHR^4, O-C_1-C_4alkylNR^4_2, -O-$(CH_2$-CH_2-$O)_mR^4$ or -O-CH_2-$C(OR^5)H$-CH_2-OR^6;

R^4 is H or C_1-C_4alkyl;

R^5 is H or C_1-C_{10}alkyl;

R^6 is H or an OH-protecting group;

m is an integer from 1 to 4;

B is a purine or pyrimidine radical or an analogue thereof.

R^{28} is H or an OH-protecting group.

37. The use of a nucleoside dimer according to claim 25 for the preparation of oligonucleotides according to claim 1.

38. The use of an oligonucleotide according to claim 1 as a diagnostic for the detection of viral infections or genetically related diseases.

39. The oligonucleotide according to claim 1 for use in a therapeutic process for the treatment of diseases in mammals including humans by means of interaction with nucleotide sequences in the body.

40. A pharmaceutical preparation comprising an effective amount of an oligonucleotide according to claim 1 on its own or together with other active ingredients, a pharmaceutical carrier and, if appropriate, excipients.

41. The nucleoside dimer according to claim 25 for use in a therapeutic process for the treatment of diseases in mammals including humans.

INTERNATIONAL SEARCH REPORT

Ir ational Application No
PCT/EP 97/03192

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H21/00 A61K31/70 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 20597 A (CIBA-GEIGY AG) 3 August 1995 see the whole document ---	1-41
X	WO 92 20822 A (ISIS PHARMACEUTICALS, INC.) 26 November 1992 see claims 1-96 ---	1,4, 8-26, 32-41
X	WO 92 20823 A (ISIS PHARMACEUTICALS, INC.) 26 November 1992 see page 1-21 ---	1,4, 8-26, 32-41
Y	EP 0 714 907 A (H.HOFFMANN-LA ROCHE AG) 5 June 1996 see abstract --- -/--	1-41

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
16 September 1997	30 -09- 1997

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Scott, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/03192

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J.LEBRETON ET AL.: "Antisense Oligonucleotides with Alternating Phosphodiester-"Amide-3" Linkages." SYNLETT, vol. 2, February 1994, pages 137-140, XP000564641 see the whole document	1-41

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/03192

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 38
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/EP 97/03192

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9520597 A	03-08-95	AU 1534795 A	15-08-95
		CA 2180727 A	03-08-95
		EP 0741741 A	13-11-96
		FI 962980 A	26-07-96
		HU 75704 A	28-05-97
		NO 963092 A	16-09-96
		ZA 9500580 A	26-07-95
WO 9220822 A	26-11-92	US 5378825 A	03-01-95
		AU 662538 B	07-09-95
		AU 1998692 A	30-12-92
		AU 666121 B	01-02-96
		AU 2150292 A	30-12-92
		BR 9206026 A	27-12-94
		BR 9206027 A	27-12-94
		CA 2103378 A	22-11-92
		CA 2103464 A	22-11-92
		EP 0586520 A	16-03-94
		EP 0586570 A	16-03-94
		HU 66378 A	28-11-94
		HU 65941 A	29-08-94
		JP 6504067 T	12-05-94
		JP 6503838 T	28-04-94
		NO 934179 A	12-01-94
		NO 934180 A	11-01-94
		US 5489677 A	06-02-96
		US 5386023 A	31-01-95
		WO 9220823 A	26-11-92
		US 5602240 A	11-02-97
		US 5610289 A	11-03-97
		US 5541307 A	30-07-96
		US 5618704 A	08-04-97
		US 5608046 A	04-03-97
		US 5623070 A	22-04-97
WO 9220823 A	26-11-92	US 5378825 A	03-01-95
		AU 662538 B	07-09-95
		AU 1998692 A	30-12-92
		AU 666121 B	01-02-96
		AU 2150292 A	30-12-92

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 97/03192

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9220823 A		BR 9206026 A	27-12-94
		BR 9206027 A	27-12-94
		CA 2103378 A	22-11-92
		CA 2103464 A	22-11-92
		EP 0586520 A	16-03-94
		EP 0586570 A	16-03-94
		HU 66378 A	28-11-94
		HU 65941 A	29-08-94
		JP 6504067 T	12-05-94
		JP 6503838 T	28-04-94
		NO 934179 A	12-01-94
		NO 934180 A	11-01-94
		US 5489677 A	06-02-96
		US 5386023 A	31-01-95
		WO 9220822 A	26-11-92
		US 5602240 A	11-02-97
		US 5610289 A	11-03-97
		US 5541307 A	30-07-96
		US 5618704 A	08-04-97
		US 5608046 A	04-03-97
		US 5623070 A	22-04-97
EP 714907 A	05-06-96	CA 2163392 A	31-05-96
		CN 1128269 A	07-08-96
		FI 955767 A	31-05-96
		JP 8208686 A	13-08-96